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FUNCTIONAL GENOMICS of ABIOTIC STRESS

GenomePrairie

FUNCTIONAL GENOMICS OF ABIOTIC STRESS AT GENOME PRAIRIE

Final Report Submitted To

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For more information, please contact

Dr. William (Bill) L Crosby
Project co-leader
Professor and Head
Department of Biological Sciences
University of Windsor
Rm 119B, Biology Building
401 Sunset Avenue
Windsor, ON, N9B 3P4
Canada
T: (519)253-3000 x2697
F: (519)971-3609
E: bcrosby@uwindsor.ca

Dr. Randall Weselake
Project co-leader
Professor,
Agricultural Biotechnology
Department of Agricultural, Food and Nutritional Science
4-10M Agriculture/Forestry Centre
Edmonton, AB, T6GP5
Canada
T: (780) 492-4401
F: (780) 492-4265
E: randall.weselake@ualberta.ca

Dr. Elizabeth Nanak
Project Manager,
Genome Prairie
Agriculture Building,
University of Saskatchewan
Room 4E24
51 Campus Drive,
Saskatoon, SK, S7N 5A9
Canada

T: (306) 966-2409
F: (306) 966-2033
E: enanak@genomeprairie.ca

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A. ABSTRACT/SUMMARY (max. 100 words)

The primary objective of the FGAS project is to understand how wheat and canola respond to the rigors of the Canadian environment at the molecular level. This will lead to a better understanding of the responses of plants to environmental stresses - heat, cold, drought and salinity. This project brings together diverse research talents from Quebec, British Columbia and the Prairie provinces into a coordinated team, devoted to attacking one of Canada's most pressing crop development issues. Knowledge gained from this project has the potential to improve the tolerance of all plants to cold and other stresses, to lengthen their growing season and to improve the quality of crops, both in Canada and around the world.

B. EXECUTIVE SUMMARY

The Functional Genomics of Abiotic Stress (FGAS) project has harnessed the skills of top plant abiotic stress researchers in Canada. They exploited a range of genomics and proteomics technologies to decipher the genetic mechanisms that underlie plant responses to a range of abiotic stresses. Two crops of importance to Canada, wheat and canola, along with the model plant *Arabidopsis thaliana*, a close relative of canola, were used as experimental systems.

The FGAS project can be described in terms of two Project Themes that organized the research around the two target crop species for the project – *Brassica* and Wheat. Each theme was in turn comprised of sub-projects that constituted the focus of research, and around which multiple Principal investigators and contributing laboratories were organized.

The FGAS project originally budgeted at $ 19.45 M had, however, secured only $15.4 M and had consequently revised its milestones according to the mid-term review recommendations. The project research and outcomes presented in this document are consistent with the mid-term re-defined objectives and milestones.

The cereal low-temperature theme

The cereal low-temperature theme has focused on elucidating novel biological mechanisms governing low temperature (LT) stress response and acclimation. The theme is organized around five sub-projects that study the functional genomics of LT tolerance, including: 1) the characterization of isogenic lines and associated resources that phenotypically discriminate multiple aspects of the LT acclimation response, 2) the exploitation of these lines for the genetic and physical mapping of selected genes using specially developed mapping populations, deletion lines, and BAC resources, and 3)defining the repertoire of genes expressed during LT acclimation in select tissues by EST sequencing analysis. These approaches were complemented by 4) proteomics studies employing the same LT acclimation regimes, tissues and genotypes. Finally, 5) target genes were validated through promoter analysis and transgenic approaches to determine the phenotype of select transgenes in transgenic crop plants. All five sub-projects were supported by a platform technology component that is responsible for strategic sequencing, proteomics, bioinformatics and microarray development. The diagram in Figure 1 outlines the interactions among the different components of the wheat component of the FGAS project, the investigators and some of the interactions with the *Brassica* group.

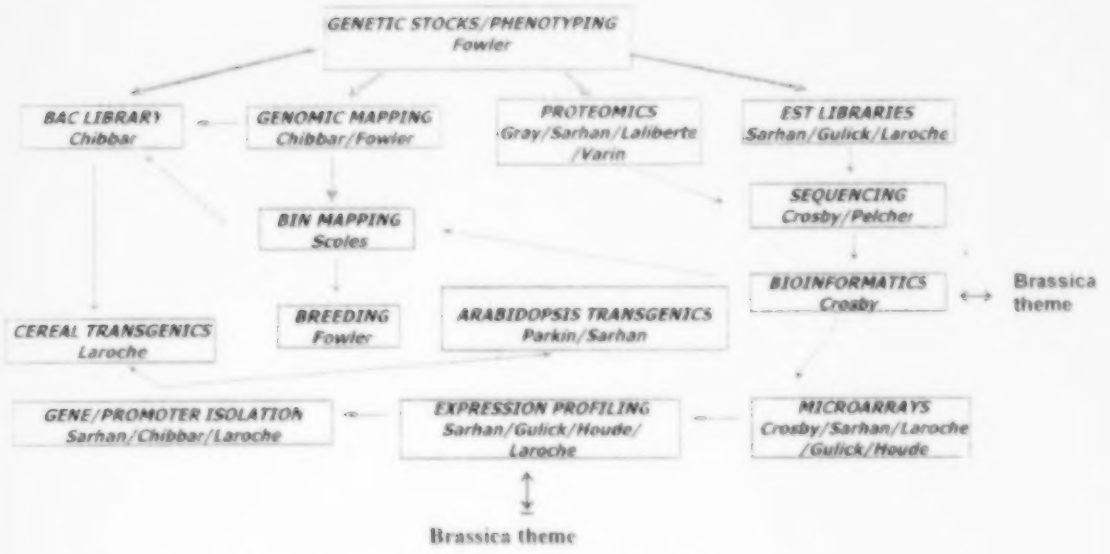

Figure 1: Interactions within the cereal low-temperature theme of FGAS

Concretely, the outcomes of the wheat team comprise:

- Development of 628 doubled-haploid (DH) lines from Norstar X winter Manitou and Norstar X Manitou (spring).
- Construction of a BAC library containing 1.27 million clones (with a coverage more than 5.5 times haploid genome equivalents) from the hexaploid cv 'Norstar' – a specialized wheat germplasm selected for cold tolerance.
- Effective completion of a specialized 82,150-run 'quality' EST data set, in support of the wheat cold-acclimation research theme, and mining of this data set for lead-candidate genes of possible importance to the regulation of cold tolerance in temperate cereals.
- Bin mapping of 600 FGAS EST clones.
- Mapping of almost 900 SSR and AFLP markers mapped on different wheat populations.
- Design of a 17,300-feature low temperature enriched oligonucleotides wheat array in collaboration with two international partners.
- Identification and characterization of candidate genes such as TaVRT-1 and TaVRT-2, antagonist genes in regulation of vernalization.
- Development of interaction maps of different wheat proteins.
- Creation of a proteomics database for low-temperature regulated chloroplastidial proteins.
- Identification of promoters for 30 genes and their functionality study.
- Identification and characterization of knockout *Arabidopsis* lines for six homologues of wheat genes.
- Development of wheat transgenic plants for nine genes of interest.

The Brassica stresses theme

The *Brassica* stresses theme has examined the effect of low temperature, drought and nutrient stress on gene expression in *Brassica* and *Arabidopsis*. This aspect of the project has developed according to the following four sub-activities: 1) novel genes controlling freezing and drought tolerance; 2) effect of abiotic stress on gene expression in microspore-derived (MD) cultures of *Brassica*; 3) proteomic and genomic analysis of abiotic stresses in roots; and 4) elucidation of the role of ABA in abiotic stress. The

diagram in Figure 2 illustrates the interactions among the different components of the *Brassica* theme and interaction with the wheat theme via bioinformatics.

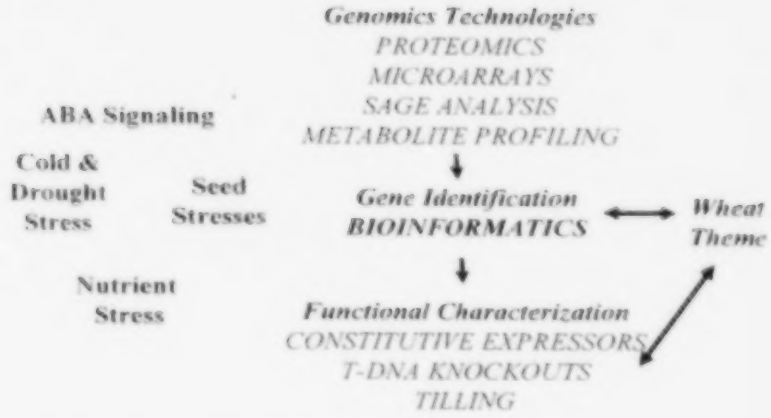

Figure 2: Interactions within the *Brassica* stresses theme of FGAS.

The achievements of the *Brassica* team include:

Cold stress

- Construction of five SAGE datasets for *Arabidopsis* (80,000 tags each). Detection of 400 *Arabidopsis* genes that are differentially regulated upon short-term cold stress.
- Printing/manufacture of a full *Arabidopsis* microarray containing more than 25,000 features from the commercially available oligos through facilities at the Univ. Alberta (Dr. M. Deyholos), with subsequent distribution of arrays and their use by select groups within the *Brassica* research theme
- Cross-species microarray data for analysis of cold acclimation in a wild crucifer.
- Proteomics data detailing proteins involved in the response of spring and winter canola to cold temperatures.
- Phosphorylation profiles for *Arabidopsis* proteins in response to low-temperature stress.

Seed Stresses

- Construction of four SSH cDNA *B. napus* libraries enriched for genes controlling the microspore-derived (MD) embryo's response to cold and osmotic stress. Clones and macroarrays will be available to the scientific community.
- Proteomics data detailing the response of MD embryos to abiotic stress.
- Microarray data uncovering gene expression profiles in response to ectopic expression of three key TFs.

Nutrient stress

- Proteomics data detailing the proteins bound to the microtubule component of the *Arabidopsis* cytoskeleton.
- Proteomics data detailing proteins present in root exudates.
- Gene expression data (microarray) in response to phosphate deficiencies.
- Genetic mapping populations for *B. napus*, segregating for a number of novel traits.
- Transgenic *Arabidopsis* lines expressing his-tagged primary metabolism proteins.

- Antibodies for a number of primary metabolism proteins.

ABA and drought stress

- Global transcript profile Global transcript profile data for Arabidopsis under drought stress and in response to ABA treatment.
- Synthesis of affinity probes with tethered ABA analogues.
- Characterization of a novel ABA binding protein.

Brassica Platform technologies

- Creation of Arabidopsis Canadian TILLING (CAN-TIL) resource.
- Testing Brassica TILLING population.
- Development of SAGE software for extracting and matching Arabidopsis SAGE tags.
- Genome Viewer for displaying genomics data against a sequenced genome.
- Implementation of an effective informatics infrastructure that permits the secure organization, analysis and exchange of data within and between project themes. This infrastructure is based on advanced open source applications that are at the forefront of Bioinformatics developments in the public domain.

To summarize, the achievements of the FGAS team include the conclusion of key elements of the research plan, the implementation of an impressive array of tools such as field genetic resources, protein analytical technologies, DNA sequence databases and related genomic resources such as microarrays, that will empower the next research phase concerned with the elucidation of biological mechanisms governing stress adaptation in Canadian crops.

The Project has also provided an opportunity to FGAS scientists to work on the most advanced scientific field of "plant genomics". The project has employed and trained 36 post-doctoral scientists/research scientists, 49 graduate/undergraduate students and 40 technical personnel. For many of the recruited personnel, the FGAS project has been a fruitful experience that has allowed them to secure permanent and/ or better research positions.

To date, the FGAS team has generated over 100 scientific contributions including 35 publications directly arising from the project; this number will increase especially as the project continues to transition from an emphasis on resource development and gene identification to functionality testing.

The project has deposited over 82,000 EST sequences at the Genbank as a contribution to the wheat international initiative. Other electronic data such as 400,000 *Arabidopsis* SAGE tags, 122 microtubule binding protein sequences were deposited respectively at the Genbank and BIND databases.

The project has established international collaborations with organizations such as the USDA network and the Australian Center for Plant Functional Genomics for the development of a 17,300-feature wheat microarray. It is expected that the number of international collaborations with FGAS researchers will increase as many genomics tools and biological material developed under the FGAS project will be available to the scientific community under collaborative agreements.

In conclusion, the FGAS project has seized the opportunity to develop a highly integrated genomics discovery platform that spans field crop genetics to advanced genomics resource and development and bioinformatics. These developments have presented the project international strategic opportunities in the area of crop genomics of direct relevance to Canada. Knowledge

gained from this project has the potential to improve the tolerance of all plants to environmental stresses, to lengthen their growing season and to improve the quality of crops of importance to Canada.

More concretely, the tools developed in the FGAS project will be exploited in the Genome Canada Competition III project "Use of Genomics Tools for Crop Improvement in Temperate Climates" for the development of molecular markers and the functionality study of proteins involved in cold tolerance. The developed knowledge will be utilized in subsequent breeding programs to improve cold resistance in cereal crops.

C. TECHNICAL REPORT

The technical report is organized as an *outline* that highlights project milestones, current progress against those milestones, amendments to the milestones (if any, and justification for same) along with a statement of objectives to be achieved by March 2005. Each section is accompanied by a brief 'Remarks and Highlights' section that raises specific achievements or issues for the attention of reviewers.

Scientific objectives and Milestones are summarized in the attached Gantt chart. The chart has been revised up to March 2006 to take into account the one year no-cost extension granted by Genome Canada.

I. CEREAL LOW TEMPERATURE STRESS THEME

The wheat theme is focused on elucidating novel biological mechanisms governing low temperature (LT) stress response and acclimation. The theme is organized around five sub-projects that study the functional genomics of LT tolerance, including: 1) the characterization of isogenic lines and associated resources that phenotypically discriminate multiple aspects of the LT acclimation response, 2) the exploitation of these lines for the genetic and physical mapping of selected genes using specially developed mapping populations, deletion lines, and BAC resources, and 3) defining the repertoire of genes expressed during LT acclimation in select tissues by EST sequencing analysis. These approaches are complemented by 4) proteomics studies employing the same LT acclimation regimes, tissues and genotypes. Finally, 5) target genes are validated through promoter analysis and transgenic approaches for determining the phenotype of select transgenes in transgenic crop plants. All five sub-projects are supported by a platform technology component that is responsible for strategic sequencing, proteomics, bioinformatics and microarray development.

Our initial research efforts have been focused on developing the research tools required to achieve our overall goal, which is to elucidate the genetic mechanisms that underlie the induction and regulation of LT tolerance at the whole plant and molecular level. We have characterized over 80,000 quality ESTs from specialized wheat libraries that have been deposited in the public domain as a strategic contribution to the international wheat genomic effort. The bioinformatics group has used this data set to design a 17,300+-feature LT enriched oligonucleotide array in collaboration with two international partners. The array has been printed successfully and he second printing set is being used by scientists from Canada, USA and Australia. These developments constitute a lead role for our array within the international wheat community. Protocols for high throughput identification of promoters and transcription factors are being developed and protein interaction maps constructed. Six hundred cDNA originating from 11 different libraries have been mapped to wheat deletion lines. We have also produced a 1.27 million clone BAC library that provides 5.5X coverage of the hardy wheat cultivar 'Norstar' genome. Three DH wheat mapping populations that have been specifically designed to assist in the discovery of key LT tolerance genes have been saturated by nearly 900 SST and AFLP markers. Application of this technology is now being interfaced with proteomics approaches to identify LT induced proteins from the chloroplast and bioinformatics analyses for an in-depth investigation of the regulatory network that determines LT tolerance.

A. GENETIC STOCKS AND PLANT RESOURCES

Objectives

- Develop mapping populations specifically designed to phenotypically discriminate select aspects of the cold acclimation response. Status: **Completed**

- Characterization of lines in mapping populations. Status: **Completed**
- Conduct both controlled-environment and field acclimation experiments involving selected germplasm for the provision of material to expression profiling (proteomics and microarray) elements of the program. Status: Controlled environment programs have been **completed**; field-level material development is **completed.** Plant tissue samples from both spring and fall field plantings of selected genotypes was collected at pre-determined sampling dates in the summer of 2003 and 2004 and stored at -80C for planned gene expression studies. Patterns of phenological development were determined for each genotype and low-temperature tolerance was measured on selected sampling dates. Status: **Completed**; second year of field sampling completed in Nov 2004. Biological material for microarray analyses was produced and collected as planned; however, critical sampling times were lost due to thawing and other difficulties during transfer between cooperating laboratories. These experiments cannot be repeated due to lack of funds.

Contributing Groups

- **Fowler** (population development, genetic analysis, physiological studies, harvest, etc.)

Remarks and Highlights

The genetic stocks available to this project were developed and initially characterized for low temperature (LT) response early in the program - indeed; much of the material was necessarily developed prior to the point at which the GC funds started to flow. Funding for this early work was provided from Ducks Unlimited Canada, SeCan, NSERC, WGRF, and the Government of Iran. Arrangements to access additional genetic stocks that have been fully characterised are being made with international partners. Extending upon prior genetic and physiological studies, field studies have been expanded under the auspices of the FGAS project. This support has been used to field validate results obtained from controlled environment studies. These studies will also expand the potential usefulness of our research to include wider agricultural applications that complement the molecular and transgenic approaches being undertaken by others.

Detailed phenotypic studies conducted to date have shown that the LT induced protective mechanisms in cereals are developmentally regulated and involve acclimation process that can be stopped, reversed, and restarted. The developmental genes are responsible for the duration of expression of LT tolerance genes while the rate of LT tolerance acquisition is determined by genotypic differences in acclimation potential. The genotypic specific genetic factors responsible for phenological development and rate of acclimation interact with environmental variables to determine LT gene expression and plant LT tolerance with the result that the full expression of LT induced genetic systems can only be observed under genotypic dependent optimum combinations of time and temperature. A high level of unexplained variation in controlled experiments and our inability to completely recover what is supposedly a single growth habit gene, *Vrn-A1*, in the production of near-isogenic lines demonstrates that detailed functional genomic or phenomic analyses will be required to isolate the critical genetic components of this highly integrated system which is regulated by environmentally responsive, complex genetic pathways. A manuscript on the interactions among the factors that regulate phenological development and acclimation rate to determine low-temperature tolerance in wheat has been accepted for publication.

B. MOLECULAR MAPPING AND BAC DEVELOPMENT

Objectives

- Map 200 ESTs per year (1,000 ESTs over 5 years) for lead candidate genes regulated by stress to the available deletion lines. **Status:** Objective of mapping 600 ESTs over the 3 year lifespan of the project; Current Status: **Completed**. The objective has been reached and final analyses have been completed.

- Screenings of doubled-haploid (DH) lines using AFLP and SSR repeat markers. Status: **Completed**; genes regulating the duration and level of phenotypic expression of low temperature have been identified and mapped.
- Construct a 5.5X coverage BAC library from cv 'Norstar', and undertake the placement of select markers and genes to the clone set. Objective (Q2, FY3): Clone approximately 10^6 independent BAC clones. Status: Completed - 1,266,432 clones picked and archived; **Objective**: **Completed** by Dec. 2003 - ahead of schedule. The library characterization has been completed and a manuscript has been published.

Contributing Groups

- **Fowler** (genetic stock provision; mapping data)
- **Scoles** (clone validation; deletion and RFLP mapping)
- **Chibbar** (marker validation; BAC development and physical mapping, SSR mapping)

Remarks and Highlights

Bin mapping:

The project target of mapping 600 ESTs was achieved by the end of March 2005. A total of 360 ESTs were successfully mapped at the CMG lab by Southern hybridization (membranes consisting of 140 lanes of *Dra* I digested DNA from nullisomic-tetrasomic (NT), ditelosomic (Dt) and single-deletion lines of Chinese Spring wheat). The remaining 240 ESTs were mapped based on homology to previously mapped ESTs in the wEST-ITEC combined sequences database of NSF (USDA). Among all of the ESTs received for mapping, 60 were determined to be unmappable using Southern hybridization due to strength of the detected signal. Suitability of the remaining ESTs was determined by initial screening using a scaled-down hybridization protocol and mini-membranes (each consisting of a single lane of Chinese Spring wheat DNA). Formatting the results of all 600 ESTs has been completed and the mapping results are currently available to group members (Figure 3). These results are available to the investigators using a FTP protocol at ftp.agr.gc.ca (userid: anonymous; password: your_email_address; when you get connected you go to /pub/outgoing/res-bg/binmap) until a web interface becomes available. A poster "Chromosome bin map of 600 cold stress responsive ESTs of wheat (*Triticum astivum* L.)" was presented at the 3rd Canadian Plant Genomics Workshop (Saskatoon, August 28th – 31st). EST mapping results are being prepared for publication in a refereed journal.

Figure 3: Chromosome Bin map of Low-Temperature Responsive EST of Wheat

Genomic Mapping:

To confirm the earlier genetic observations that a cold-tolerance related locus (loci) is present on chromosome 5A, but it is genetically distinct from *Vrn-1*A, several new markers located on chromosomal regions previously not associated with cold tolerance have been authenticated. A total of 628 doubled haploid (DH) lines for three related mapping populations have been screened for cold tolerance (LT_{50}). The LT_{50} values of these populations ranged from the less-hardy parent to the hardy parent. One mapping population that has been fully characterized for LT_{50} consists of 264 selected lines from a cross between the hardy Norstar (winter) and the less hardy Cappelle-Desprez (winter) where both parents carry the winter habit locus *vrn-A1*. The second mapping population that has been fully characterized for LT_{50} consists of 143 lines selected from the cross between Norstar and the less hardy winter Manitou line. The winter Manitou parent is an isogenic line that carries the genetic background of the spring wheat cultivar, Manitou, and the winter habit locus (*vrn-A1*) from Norstar. The third population is derived from the Norstar / Manitou cross (221). A total of 452 SSR and 443 AFLP markers, have been mapped against lines from these populations. A consensus genetic map has been constructed for the winter wheat Norstar.

Further genetic analysis revealed that LT tolerance quantitative trait loci (QTL) spanned a > 30 cM large region with a peak located proximal to the *vrn-A1* locus. The genetic mapping data in combination with cDNA-AFLP (RNA finger printing) has also been used to identify transcripts associated with early stages of cold-tolerance induction in wheat. More than fifty percent of the genomic mapping work is supported by funds obtained through competitive grants by Brian Fowler and Ravi Chibbar and funds from industrial partners. The number of DH lines was increased to 628 lines as instructed by the Genome Canada Site Visit Committee.

BAC Library:

A 1,266,432 clone BAC library was in place by December 2003. The library has been characterized and a manuscript was published in Crop Science. Screening one-seventh of the BAC library with radiolabeled CBF resulted in the identification of 27 positive BAC clones.

From these 27 positive BAC clones, Southern blot analysis was used to identify two positive BAC clones which carry the CBF clones participating in low-temperature tolerance. DNA sequencing confirmed that the identified BAC clones carried CBF genes. Detailed sequence analysis is currently under way to characterize the BAC clones carrying CBF genes. More than ninety percent of the research related to BAC library construction and characterization was carried out with funds other than GP. Drs. Fowler and Chibbar obtained these funds as NSERC-NRC collaborative grant holders with industrial partners Secan, WGRF and Ducks Unlimited. As requested by the SMAC, options for a sustainable plan for preservation and distribution of the BAC library to ensure availability to Canadian and International scientists will be examined.

C. EXPRESSION ANALYSIS - ESTs AND MICROARRAY DEVELOPMENT

Objectives

- Construct cDNA libraries using mRNA prepared from tissues undergoing stress acclimation and qualify these for utility in an EST sequencing program by FY1. Status: **Completed**
- Develop and carry out suppressive subtractive hybridization (SSH) and virtual subtraction (VS) strategies for increasing the clone diversity of the EST sequencing program by FY2. Status: **Completed**
- Derive 80,000 high quality EST sequences from stress cDNA libraries by the end of FY2. **Completed** as of Dec 3rd 2003; >80,000 high quality sequences were deposited to GenBank.
- The proposed EST sequencing program for wheat has been completed to the extent and to a quality standard defined by the Mid-Term Review process in June of 2003. This aspect of the program was completed 3 months ahead of schedule.
- In response to a recommendation by the Site Visit Committee, the group has been tasked with fully sequencing those cDNAs found to be 'unique' vis-à-vis the public data sets. Status: **Terminated**: Discussions ensued and a work plan was established. However, due to financial constraints coupled to the ongoing activities of Dr. Yasunari Ogihara, Japan, who has made a large commitment to sequencing full-length wheat cDNAs, it was agreed to terminate this goal. As the results from the Japanese group are not yet available in public databases, it was felt that the high risk of sequence redundancy was not worth the financial commitment necessary to complete this portion of the project.
- Organize and analyze, on an ongoing basis, the sequence data for the development of microarrays, by FY2. Status: **Completed**; A high quality microarray of 17,300 has been successfully printed. Quality control of the first printed set was deemed as highly satisfactory by Canadian, US and Australian scientists. Slides from the second printing set are being actively used by these scientists. Signal intensity remains an issue not resolved yet. Functional annotation and documentation for probe identification has been prepared in Montreal and is ready for release.
- Undertake a survey of EST data to select candidate low-temperature regulated genes for further analysis. **Objective** (Aug 2003) Identify 100 candidate genes based on motif content (AP2, MADS, bHLH, etc.) and comparative information during stress exposure (in *Arabidopsis* and Rice); Status: **Completed,** over 1000 candidate regulatory genes have been identif[illegible]. Future goals (March 31, 2005); identify stress regulated candidate genes using microarray for analysis. Status: **Ongoing**, (see above) Data is being generated by PI in Montréal and Lethbridge.

Contributing Groups

- **Fowler** (input plant material and physiology data)
- Sarhan (library generation and qualification, gene discovery)

- Houde (gene discovery)
- Gulick (virtual subtraction, gene discovery)
- Laroche (SSH library generation and qualification, gene discovery)
- **Crosby** (high-throughput sequencing and bioinformatics)

Remarks and Highlights

cDNA libraries from 11 different tissues and cold treatment regimes have been prepared and samples forwarded for EST sequencing. Efforts were made to obtain full-length sequences using both anchored priming on poly-A tail and 5' end cap structure in order to minimize any bias from a single approach. The 5' single-pass sequences show the presence of a deduced in-frame ATG across a range of gene size classes suggesting that a large portion of the inserts represent full-length sequences.

The 82,150 quality (PHRED > 20) sequence define a total of about 30,978 distinct sequences (current UniGene set). The comparative frequency of singletons within the total versus UniGene datasets for the FGAS data set is 16% and 42% respectively - a value very similar to the TIGR wheat Gene Index (15% and 63%).

The 60,000 VS sequences have been surveyed for predicted regulatory genes (e.g. transcription factors, protein kinases, E3 ubiquitin ligases) from which 1,240 non-duplicate candidates were identified. These are priority candidates for further transcription profiling, promoter analysis and mapping studies.

The Bioinformatics group has used quality-filtered EST data for the design of an initial 10,000-feature MIAME-compliant micro-array using 70-mer oligonucleotides. The FGAS project has catalyzed the formation of a multinational consortium, initially involving the Australian Plant Functional Genomics Centre (Dr. Peter Langridge, CEO), whose members will financially co-support the development of the initial 10,000-feature set, with a view to extending the set to include an additional 10,000 features. We have designed an additional 7000 probes that were synthesized with collaboration and co-support with Dr. Olin Anderson, USDA ARS, Albany, CA. This is the base of production of our current 17,300 feature array. Initial trial printing of the arrays by Microarrays Inc., Nashville TN, was successful and production of the array is now available and has been used by scientists from Canada, U.S.A. and Australia although a general weak signal has been reported. Trouble shooting is continuing but at a reduced rate.

The identification of candidate genes involved in the regulation of cold tolerance is on-track, with the expression (Northern blot) profiling of over 100 genes completed to date. The AP2 (133 different genes predicted based on contig assembly of available ESTs), MADS (80 different genes predicted), bHLH (ICE, Inducer of CBF Expression; small gene family), FLA-like AGPs (63 genes predicted), and RNA binding proteins full length cDNAs are being isolated. So far, 128 full length members (less than 97% identity) of the AP2 family have been isolated and sequenced. Thirty five of these genes belonging to the CBF group are subdivided into 15 subgroups compared to 6 subgroups for rice and *Arabidopsis*. Using northern blot analyses and Real Time-PCR, all subgroups were shown to be inducible by LT exposure. The promoters of 4 CBF genes were isolated and 4 CBF genes were subcloned in the pENTR vector for protein expression. These proteins will be used for binding studies to COR promoters and for antibody production. They will be useful for immunoprecipitation studies to identify interacting proteins since the two hybrid studies do not function with these proteins (possibly due to highly acidic regions in the CBF protein that are able to act as activation domain). For the MADS family, we have isolated and sequenced 48 full length cDNAs. Twenty five of these were mapped by the FGAS project to wheat chromosomes. Preliminary phylogenetic analyses indicate that these proteins contain homologs of several genes involved in the control of *Arabidopsis* flowering. The TaVRT-2 and VRN2 (zinc finger) proteins were produced for promoter binding studies and antibody production. To determine if some of these proteins can interact with the TaVRT-1 or TAVRT2 promoters, in vivo transactivation assays have been performed using GFP as a reported gene. The results demonstrate that the CBF1 protein interacts with the TaVRT2 promoter and enhances its activity. On the other hand, the results suggest that the binding of TaVRT2 protein to the TaVRT1 promoter is required, but not sufficient for its transrepression. The DNA protein complex containing TaVRT2 and TaVRT1 promoter may require another protein, probably VRN2 that functions as

a transrepressor. The transactivation assays with TaVRT2/VRN2 proteins and TaVRT1 promoter have been initiated. The preliminary results showed that the VRN2 protein doesn't affect TaVRT2 promoter activity, but TaVRT2 and VRN2 proteins, together, repress its activity. Transactivation assays with ICE proteins and CBF promoter will be initiated. These results demonstrate the molecular links between flowering control and low temperature. In addition, the expression of four candidate genes which are presumed to be associated with flowering were examined by semi-quantitative RT-PCR using Norstar and Manitou wheat and their near-isogenic lines. The expression of the SOC-like and SVP-like MADS box genes showed no difference between the two genotypes. However, the expression of the SOC-like gene was slightly increased during cold acclimation. The FLOWERING LOCUS T (FT-like) gene was induced after the vernalization saturation point in winter wheat, while in spring wheat the induction occurred after 1-2 weeks of cold acclimation. The expression of the CONSTANS (CO)-like gene decreased gradually and increased again around 5-6 weeks of cold acclimation in winter wheat, while it was nearly constant in spring wheat. Another MADS protein, AGL20-like, has been expressed in bacteria to be used in DNA-binding assays. These genes are being targeted for protein interaction studies in the coming quarter. We are also near completion of optimizing several variables for in situ hybridization experiments such as fixing of tissues and hybridization of probes. In the current quarter, these experiments will start allowing us to determine whether mRNAs corresponding to interacting partners are expressed in the same tissues and developmental stages. The FLA-like AGPs encode proteoglycans that contain both fasciclin and AGP domains with most of them predicted to have a glyphosylphosphatidyl-inositol (GPI) anchor that links them to the cell surface. Several recent reports showed that these proteins play important roles in various aspects of plant growth and development including responses to abiotic stresses. Currently, there is no detailed characterization available of this gene family in wheat (or cereals in general). Among the 63 predicted FLA-like AGPs genes in wheat, we have so far obtained the full length sequence of 27 members of this family (~50%). So far 10 % of these genes are regulated by abiotic stresses. Genetic mapping of some of these wheat FLAs is currently underway. We have recently identified 5 different members of RNA binding proteins (full sequence completed). Transgenic *Arabidopsis* plants overexpressing a cold regulated member were produced and phenotypic analyses will be available soon. In addition, this gene was expressed in bacteria, and the purified protein will be used in binding studies to determine if it has any specificity with any known *cor* genes. Two distinct bHLH ICE homologs have been expressed with HIS tags and affinity purified for promoter binding studies with three different myc fragment homologs (from the wheat CBF227 promoter). Ice 41 was found to bind to a myc2 homolog and ICE 87 was found to bind strongly with a myc3 homolog and with less efficiency with a myc4 homolog (core sequence similar to myc3). In vivo transactivation assays using the CBF227 promoter has shown a strong activation with ICE41 and a very strong activation with ICE87.

The Concordia program has identified over 300 signal transduction candidate genes that are induced or repressed during cold acclimation. The candidates include transcription factors, receptor kinases, protein kinases, protein phosphatases, RNA binding proteins, G-nucleotide regulatory protein, calcium binding proteins, E3 ubiquitin ligases. More than 120 of these candidates show significant differences in regulation in winter and spring wheat and are the focus of further characterization. A manuscript on transcript profiling for these genes is in preparation. Expression profile analysis of smaller set of cold regulated genes has been published in Genome.

A number of candidate regulatory genes have been characterized in depth. The isolation of full length cDNA clones for cold acclimation induced receptor kinases F29A and F29B was completed. RT-PCR characterization confirmed cold regulation. Yeast 2-hybrid screen identified specific interaction of F29A interaction with one member of the alpha-tubulin family, a principal component of microtubules, glyoxylase I and a third cold-regulated protein of unknown function. Characterization of the F29 gene family in wheat cultivars that differ in cold tolerance is completed and localization studies are in progress and a manuscript is in preparation. The fifteen members of the alpha-tubulin gene family in *T. aestivum* has been characterized for cold regulation and a manuscript is in preparation. The full-length cDNA encoding the cold-induced small GTP binding protein, J822 has been identified and it's regulation by other environmental stresses and signalling compounds is completed. J822 has been shown to interact

with several proteins most significantly with two phospholipase C's , which have have also been shown to be cold regulated (manuscript in preparation). In addition, the cold regulated J900, a calcium binding EF-hand protein, was found to interact with a G-protein alpha subunit.

The Lethbridge program has used a microarray (amplicons) of about 4800 wheat sequences related to disease and 800 rye sequences related to low temperature to identify differentially regulated sequences and develop their abilities to use microarrays. Hybridization with 2 independent biological sets of probes revealed that 97 sequences were up regulated and 247 down regulated by a factor of 2 fold or more. When using a factor of 4 fold or more, 25 sequences were up regulated and 17 down regulated. Of these 21 sequences of known and unknown functions are being further characterized using real-time (RT) PCR. Additional hybridization to the array with different cultivars/lines is on going. A 480-feature cDNA amplicon array containing putative transcriptions factors and other genes was derived from the TaLt library data in cooperation with Dr. Mike Deyholos (U. Alberta, Edmonton). The array has been hybridized with three different set of biological samples with dual technical replicates with RNA sampled after 12h, 24h, 7d and 21d from cultivars PI178383, CI14106 and Norstar. This has led to the identification of 18 candidate genes from which 16 (transcription factors from 6 different families, (WD40, ER, ERD6, BTF3, ZF and WRKY), translation factors (eIF1a, eIF3, eIF4a3) and one DNA binding protein (HMG) are being further characterized using real time PCR. We have fine tuned microarray experiments as various components were verified , e.g., comparisons of different extraction methods of RNA, polyA and total RNA labelling efficiency, direct and indirect labelling of cDNA probes, cleaning up labelled probes, as well as different parameters during the analysis. RNA samples from different cultivars and subjected to different low temperature treatments were isolated and stored for analyses of microarrays. Different conditions (e.g. different hybridization solutions, temperature, washing stringencies) were also tested for optimum hybridization to the oligonucleotide array and an optimal protocol adopted. With the availability of the GeneChip® Wheat Genome Array, preliminary work with a collaborator has been initiated to optimize hybridization of this new Affymetrix array. Replicated RNA samples have been isolated and polyA+ RNA obtained for different temperature treatments and wheat cultivars. These mRNA are ready for making probes for labeling the arrays. Given the substantial cost of the Affymetrix array, mRNA from fewer sampling points and cultivars will be tested. These specific samples points will be based on preliminary results using the FGAS 17.3K array. The FGAS 17.3K microarray printed by Microarray Inc. was received and evaluated. This is a high quality array and hybridization experiments with different treatment and three cultivars have been completed. Preliminary analyses based on sequence differentially regulated by 2 or more standard deviation units indicate that more than 450 genes were differentially regulated in Norstar and PI 178383. These genes were identified in more than 15 different functional groups including transcription factors. A subset of these genes is being validated using RT-PCR. The data is being re-analyzed using clustering algorithms. The question about signal intensity of the hybridized spots and the percentage of hybridized spots remains.

We have also received a new cDNA array of 7,000 elements duplicated that contains 650 FGAS sequences including the 480 transcription and translation factors from the TransFac array. The larger number of wheat sequences will enable a better establishment of the base line and minimize sample variation previously observed with the TransFac array. Analyses of results are being completed. Expression profiling of stress-regulated genes will be confirmed by northern and/or Real-time PCR. Preliminary analyses based on sequence differentially regulated by 2 or more standard deviation units indicate that more than 220 genes including 160 different transcription factors were differentially regulated in lines CI 14106, PI 178383 and DH+268. Differentially regulated transcription factors belongs to 12 different groups bases on identification of functional domains and the other genes belongs to 11 different functional groups. The regulation of these genes was quite different among the three lines. These results also confirmed the hypothesis that a larger number of wheat sequences present on the ECORC array would enable a better establishment of the base line and minimize sample variation previously observed with the TransFac array was shown to be true. Expression profiling of a subset of these stress-regulated genes has been validated using RT-PCR while a second subset is being verified. The data is being re-analyzed using clustering algorithms. Analyses of results are being completed.

Over 30K sequences clones from the 6 TaLt SSH cDNA libraries were preserved on CloneSaver Cards.

Three low temperature-regulated non-specific lipid transfer proteins from the ns-LTP1 family (ca. 9 kDa and all possessing coding sequences of 348 bp) and containing four pairs of conserved disulfide bonds were expressed into *Pichia pastoris* strain GS115. All three clones were expressed and purified by affinity from the media. Expression of the fused LTP1s exhibited the expected molecular weight as confirmed by western blotting. In vitro lipid binding activity for all ns-LTP1s was demonstrated and these ns-LTP1s differentially inhibited spore germination and mycelial growth of 11 fungal plant pathogens demonstrating that these three different ns-LTP1s were differentially toxic to a wide range of plant pathogens and may be involved in specific defence functions in wheat. A manuscript is being prepared.

D. PROTEIN INTERACTION AND PROTEOMICS

Objectives

- An interaction chain of some 68 proteins, totaling 86 binding pairs have been generated (see figure 1). We have demonstrated that ICE87 homodimerizes as well as heterodimerizes with ICE41, and that ICE41 also homodimerizes. Moreover, interaction of the Receptor-like protein kinase (F2965) is highly specific for one type of alpha tubulin. We have confirmed by Bi-molecular fluorescence complemention (BiFC) in *Nicotiana benthamiana* that TaVRT-1 interacts with TaVRT-2, 57H08, 31K05, VRN-2, Elongin, Immunophilin and the Pathogenesis related protein 1; TaVRT-2 with VRN2 and Immunophilin; Immunophilin with the Pathogenesis related protein 1. Status: Ongoing. (March 2006) Confirm selected interactions by BiFC. Interactions to come: TaVRT-2 with abcissic acid responding protein/LEA; TaVRT-1 with Cytochrome P450-like protein; TaTILA with Lipid transfer protein 3; and Receptor-like protein kinase (F2965) with alpha-tubulin
- Develop protocols for enrichment of plasma membrane, chloroplast, nuclear and mitochondrial fractions from control and cold-treated tissues. Status: **Completed**
- Evaluate competing protocols for the large-scale acquisition of proteomics data, particularly 2-D gel based methods, versus high-throughput MUDPIT fractionation methods suitable for MS-based analysis. Status: **Completed**
- Identify and characterize proteins in organelle fractions by MS analysis, including relative abundance measurement in control versus cold-treated samples. In total, this will involve the submission of 29 pair wise samples (chloroplastic and mitochondrial) for ICAT-MUDPIT analyses. To date, 8 samples have been submitted and arrangements have been made for additional sampling. Status: **Completed.** Mitochondrial studies have been postponed.
- **Objective** (September 2004): Complete MS studies involving chloroplast and mitochondrial fractions by Sept 2004. This will involve submission of the additional samples indicated above. Status: **Completed.** Replicate samples submitted with new samples as indicted below. Mitochondrial studies have been postponed.
- **Objective** (September 2004): Initiate detailed molecular studies examining select chloroplastic and mitochondrial protein targets identified from above. Status: **Ongoing.** Mitochondrial studies have been postponed according to the mid-term review.
- **Objective**: Complete MS studies involving chloroplast and nuclear fractions. Status: **Ongoing.** Extended to include additional sampling (See below). Analyses of nuclear fractions have been terminated due to technical problems.
- **Objective** (March 2004): Complete MS studies involving plasma membrane and mitochondrial fractions by March 2004. This will involve submission of 10 additional mitochondrial fractions. Status: **Postponed.** All plasma membrane samples are available but analyses have been postponed. Mitochondrial studies have been postponed according to the mid-term review.

- **Objective** (June 2004) Two new sampling dates planned for chloroplast isolation for non-acclimated and cold acclimated plants using two near isogenic lines and their parent backgrounds under both long and short day photoperiods. Status: **Completed**
- **Objective** (March 2005) Initiate MS analysis of new chloroplast samples and replicates of first sampling using a newly developed differential labeling technique which promises to be more robust, tagging all primary amines in a mixture (in combination with UVic-Genome BC Proteomics Centre). Status: **Ongoing**, funding for the additional analysis of samples at the UVic Genome-BC Proteomics Centre was restored. Samples, 13 in total, from isogenic wheat lines grown under various temperature and photoperiod regimes were sent for analysis in mid-March. These samples included replicates of one time point from the first sampling. Preliminary results were received in June 2005. Status: **Completed** for preliminary results.
- **Objective** (March 2006) Complete MS analysis of new chloroplast samples and replicates of first sampling. Status: **Ongoing**
- **Objective** (March 2006) Initiate detailed molecular studies examining select chloroplastic protein targets identified from above in isogenic lines. Status: **Pending**

Contributing Groups

- **Gray**/Sarhan (fractionation protocol optimization, preparation of large-scale organelle fractions, MS data analysis)
- Laliberté/Sarhan/Gulick (yeast two-hybrid libraries, screening and protein interaction studies)
- **Fowler** (input lines, physiology studies, plant material provision)
- **Crosby**/Sarhan (Bioinformatics)

Remarks and Highlights

After assessing issues of relative throughput, sensitivity, overall cost effectiveness and amenability to comparative quantitation studies, the group concluded that ICAT-MUDPIT fractionation upstream of MS analysis would more quickly and cost-effectively serve the goals of the FGAS project. The FGAS project has therefore partnered with the University of Victoria (BC) and accessed the Genome BC Proteomics Platform. In order to expedite the optimization of protocols vis-à-vis the ICAT-MUDPIT MS interface to the project, and to amass sufficient material for study, the group has focused its attention on nuclear and chloroplast fractions, until such time as the MS analysis pipeline and subsequent data analysis is operating smoothly. Material from control and cold-challenged plants across a 5-point time series was collected. Nuclear, as well as chloroplastic fractions were purified and sent for ICAT-MUDPIT analyses. Partial datasets have returned and are currently being examined and only 42 nuclear proteins were revealed for the 35 days cold-acclimated/non-acclimated pair. Several potential problems were suggested by U. Victoria. However, the samples were provided in sufficient quantity (Bio-Rad DC assay and SDS-PAGE analyses) and quality (typical nuclear proteins pattern; efficient trypsin digestion).

Coordination with Bioinformatics group (Crosby) to generate a database for peptide identification has been completed in Gray's laboratory. Re-analyses of ICAT-MUDPIT data (8 samples) using filtered database has been completed. Initial datasets have been deposited to FGAS website. The full data set from the entire time course for the chloroplastic samples is currently under analysis and a manuscript is in preparation based on initial/reanalyzed datasets. Technical difficulties with ICAT led the scientist to opt for a different labeling approach (iTRAQ.)

Preliminary data from iTRAQ labeling received and results look promising. Comparisons were made between cold acclimated and non-acclimated winter and spring wheat samples. Several known cold induced proteins were identified, providing a positive control for the experiments. The remaining samples are currently being processed at the UVic facility with completion expected by December 2005

When plants are shifted to low temperature a feedback inhibition of photosynthesis initially occurs, primarily due to Pi limitation. However, development at low temperature results in a recovery of photosynthesis in cold tolerant plants such as cereals. This is a result of a reprogramming in

photosynthetic carbon metabolism, with winter cultivars showing a greater ability for this readjustment as opposed to spring cultivars. This is supported by studies demonstrating that freezing tolerance is directly correlated to photosynthetic capacity.

Vernalization is defined as the initiation of flowering in response to low temperature and reflects the developmental transition from the vegetative phase to the reproductive phase in winter cultivars. The vernalization requirement is controlled, in part, by the Vrn-A1 gene. Cereals with the dominant Vrn-A1 allele display spring like characteristics and are usually defined as spring cultivars, such as Manitou. In contrast, cereals with the recessive vrn-A1 allele display the winter characteristics and are usually defined as winter cultivars, such as Norstar. The near-isogenic 11W line represents a unique source of genetic material as it contains the genetic background of spring wheat (Manitou,) with the recessive allele of vrn-A1. Based on freezing tolerance determinations and developmental studies, 11W represents a spring wheat made into a winter wheat (Limen and Fowler). By utilizing the 11W line our goal was to examine the relationship between cold acclimation and vernalization at the level of photosynthesis.

The data in Table 1 indicate that in winter wheat an increase in many of the proteins involved in photosynthetic carbon metabolism occurs in response to cold acclimation. It also appears this is accompanied by a down regulation in photosynthetic electron transport components (Table 1). In contrast, cold acclimation of spring wheat resulted in a down regulation of carbon metabolism enzymes and a slight up regulation in proteins associated with photosynthetic light-harvesting (Table 2). These results are consistent with those previously reported based on physiological and biochemical measurements. One interpretation is that this spring cultivar is attempting to increased photosynthetic capacity but is limited at the level of carbon fixation.

Since the near-isogenic line 11W behaves like a winter wheat in terms of freezing tolerance, it raises an interesting question in terms of how it will respond photosynthetically. Our proteome analysis presented in Table 3 indicates that 11W behaves like a true winter wheat with respect to modulation of photosynthesis. These data suggest a specific role for photosynthetic function in the cold acclimation process which differs between spring and winter cultivars of wheat. In addition, it also points towards a role for these processes in the development and vernalization processes.
Additionally, in all comparisons examined, an increase in proteins known to be associated with cold acclimation was evident (Tables 1-3). These observations acted as a positive control and further validate our findings.

Further analyses of multiple pair wise comparisons of these genotypes and others grown under different temperature (20 and 5°C) and photoperiod (16 and 8 hour) regimes is currently under way by an MSc bioinformatics student.

Table 1: Comparison of non-acclimated and cold acclimated winter wheat (*Triticum aestivum L.* cv Norstar). Ratios >1 indicate a higher abundance of proteins in the non-acclimated material while ratios <1 indicate a greater abundance of proteins in the cold acclimated material.

Accession	Protein Name	Ratio 115:114
F2NUD1	photosystem II protein D1 precursor - rye chloroplast	1.6911
PSBE_HORVU	Cytochrome b559 alpha subunit (PSII reaction center subunit V). - Hordeum vulgare (Barley)	1.625
A32364	photosystem I iron-sulfur protein - barley chloroplast	1.413
FERZA	photosystem I iron-sulfur protein psaC - rice chloroplast	1.413
S16260	photosystem II oxygen-evolving complex protein 1 - common wheat x Sanduri wheat	1.3692
F2WTH	photosystem II phosphoprotein psbH - wheat chloroplast	1.3627
F1BH4	photosystem I chain IV precursor - barley	1.2815
Q8S6F7_ORYSA	Putative PSI P700 apoprotein A2 from chromosome 10 chloroplast insertion - Oryza sativa (japonica cu	1.2488
Q5N997_ORYSA	Chloroplast photosystem I P700 apoprotein A2 - Oryza sativa (japonica cultivar-group)	1.2488
A2RZP7	photosystem I P700 apoprotein A2 - rice chloroplast	1.2488
Q6QY58_ORYSA	Photosystem II 47 kDa protein - Oryza sativa (indica cultivar-group)	1.2211
S20937	photosystem I chain V precursor - barley	1.2206
PSBH_ORYNI	Photosystem II reaction center H protein (Photosystem II 10 kDa phosphoprotein) (PSII-H) - Oryza niv	1.1813
S14962	plastoquinol-plastocyanin reductase (EC 1.10.99.1) 17K protein - wheat chloroplast	1.1784
Q7XYD3_WHEAT	Oxygen-evolving complex (Fragment) - Triticum aestivum (Wheat)	1.1548
S07296	plastoquinol-plastocyanin reductase (EC 1.10.99.1) cytochrome f precursor - wheat chloroplast	1.0903
Q9AU61_WHEAT	Violaxanthin de-epoxidase - Triticum aestivum (Wheat)	0.8322
Q8H764_WHEAT	Zeaxanthin epoxidase (Fragment) - Triticum aestivum (Wheat)	0.7772
Q9FEE4_WHEAT	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit - Triticum aestivum (Wheat)	0.6057
O22387_ORYSA	Glyceraldehyde-3-phosphate dehydrogenase (Fragment) - Oryza sativa (Rice)	0.5516
T06176	ribulose-bisphosphate carboxylase activase (EC 6.3.4.-) A2 - barley	0.5
TVWTGC	phosphoglycerate kinase (EC 2.7.2.3) precursor, chloroplast - wheat	0.4579
P93608_WHEAT	Cold acclimation protein WCOR410c - Triticum aestivum (Wheat)	0.455
Q9ATG4_WHEAT	Lipid transfer protein precursor - Triticum aestivum (Wheat)	0.2296
T06812	cold acclimation protein WCOR615 - wheat	0.2108
Q9SDV6_WHEAT	Cold-responsive protein COR14a (WCOR14a) - Triticum aestivum (Wheat)	0.1914
Q06540_WHEAT	Cold acclimation protein WCS19 - Triticum aestivum (Wheat)	0.1585
Q8S9H0_WHEAT	Temperature stress-induced lipocalin - Triticum aestivum (Wheat)	0.1302
Q8S387_WHEAT	WCOR14c - Triticum aestivum (Wheat)	0.1027

Table 2: Comparison of non-acclimated and cold acclimated spring wheat (*Triticum aestivum L.* cv Manitou). Ratios >1 indicate a higher abundance of proteins in the non-acclimated material while ratios <1 indicate a greater abundance of proteins in the cold acclimated material.

Accession	Protein Name	Ratio 116:114
Q9FRZ5_WHEAT	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit.- Triticum aestivum (Wheat).	1.5864
Q6XW16_WHEAT	Ribulose-1,5-bisphosphate carboxylase activase (Fragment).- Triticum aestivum (Wheat).	1.3662
Q7YKX2_WHEAT	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit.- Triticum aestivum (Wheat).	1.1784
T03679	probable fructose-bisphosphate aldolase (EC 4.1.2.13) precursor, chloroplast - rice	1.1443
S07296	plastoquinol-plastocyanin reductase (EC 1.10.99.1) cytochrome f precursor - wheat chloroplast	0.9872
Q8RVZ8_WHEAT	Ferredoxin-NADP(H) oxidoreductase.- Triticum aestivum (Wheat).	0.9857
Q943K1_ORYSA	Putative photosystem II subunit PsbS.- Oryza sativa (japonica cultivar-group).	0.9567
PSBH_WHEAT	Photosystem II reaction center H protein (Photosystem II 10 kDa phosphoprotein) (PSII-H).- Triticum	0.9419
S51813	photosystem-I PSI-F chain precursor - barley	0.9281
Q6QXU8_ORYSA	Photosystem I P700 apoprotein A2.- Oryza sativa (japonica cultivar-group).	0.9033
O22543_ORYSA	Chlorophyll a-b binding protein.- Oryza sativa (Rice).	0.8706
Q8RVZ9_WHEAT	Ferredoxin-NADP(H) oxidoreductase.- Triticum aestivum (Wheat).	0.8246
Q943W1_ORYSA	Putative 33kDa oxygen evolving protein of photosystem II.- Oryza sativa (japonica cultivar-group).	0.7904
WMRZ17	plastoquinol-plastocyanin reductase (EC 1.10.99.1) 17K protein - rice chloroplast	0.7863
Q5Z5A8_ORYSA	Putative photosystem II stability/assembly factor HCF136, chloroplast.- Oryza sativa (japonica culti	0.7619
Q7X9A6_WHEAT	Putative Rieske Fe-S protein.- Triticum aestivum (Wheat).	0.7287
S10561	chlorophyll a/b-binding protein CP24, photosystem II - barley (fragment)	0.7253
Q6ZG03_ORYSA	Hypothetical protein OJ1118_F05.12.- Oryza sativa (japonica cultivar-group).	0.7217
S05012	photosystem I protein psaH precursor - barley	0.7143
S38255	plastocyanin precursor - barley	0.6963
CDBH3	chlorophyll a/b-binding protein type III precursor - barley	0.6896
Q9SDV5_WHEAT	Cold-responsive protein COR14b.- Triticum aestivum (Wheat).	0.5554
S21386	chlorophyll a/b-binding protein CP29 precursor - barley	0.5454
P93608_WHEAT	Cold acclimation protein WCOR410c.- Triticum aestivum (Wheat).	0.3553
Q06540_WHEAT	Cold acclimation protein WCS19.- Triticum aestivum (Wheat).	0.3175
S07474	early light-induced protein, low molecular weight, precursor (clone HV90) - barley chloroplast	0.146
Q8S386_SECCE	REP13.- Secale cereale (Rye).	0.1169

Table 3: Comparison of non-acclimated winter wheat (*Triticum aestivum L.* cv Norstar) and the cold acclimated 11W line. Ratios >1 indicate a higher abundance of proteins in the non-acclimated material while ratios <1 indicate a greater abundance of proteins in the cold acclimated material. 11W is a near-isogenic line containing the genetic background of spring wheat (Manitou) with the recessive allele of vrn-A1. This effectively makes the spring wheat a winter wheat based on freezing tolerance and developmental studies.

Accession	Protein Name	Ratio 115:117
F2NUD1	photosystem II protein D1 precursor - rye chloroplast	2.0174
S22763	photosystem II oxygen-evolving complex protein 2 precursor - wheat	1.8296
F2WTH	photosystem II phosphoprotein psbH - wheat chloroplast	1.7545
PSBH_MAIZE	Photosystem II reaction center H protein (Photosystem II 10 kDa phosphoprotein) (PSII-H).- Zea mays	1.7545
A32364	photosystem I iron-sulfur protein - barley chloroplast	1.7378
S16260	photosystem II oxygen-evolving complex protein 1 - common wheat x Sanduri wheat	1.5714
S35159	photosystem I chain psaN - barley	1.5377
PLAS_HORVU	Plastocyanin, chloroplast precursor.- Hordeum vulgare (Barley).	1.4379
S38255	plastocyanin precursor - barley	1.4379
S14962	plastoquinol-plastocyanin reductase (EC 1.10.99.1) 17K protein - wheat chloroplast	1.2738
P82339_PEA	Putative photosystem I reaction center subunit PSAN (PSI-N) (Fragment).- Pisum sativum (Garden pea	1.1663
Q5N997_ORYSA	Chloroplast photosystem I P700 apoprotein A2.- Oryza sativa (japonica cultivar-group).	1.165
Q9LLD7_AVESA	Fructose 1,6-bisphosphate aldolase (EC 4.1.2.13).- Avena sativa (Oat).	0.8577
TVWTGC	phosphoglycerate kinase (EC 2.7.2.3) precursor, chloroplast - wheat	0.8336
Q5Z5A8_ORYSA	Putative photosystem II stability/assembly factor HCF136, chloroplast.- Oryza sativa (japonica culti	0.7587
Q7YKX2_WHEAT	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit.- Triticum aestivum (Wheat).	0.758
B96597	Rubisco subunit binding-protein beta subunit [imported] - Arabidopsis thaliana	0.7516
Q943K1_ORYSA	Putative photosystem II subunit PsbS.- Oryza sativa (japonica cultivar-group).	0.7278
O22387_ORYSA	Glyceraldehyde-3-phosphate dehydrogenase (Fragment).- Oryza sativa (Rice).	0.7251
T02071	glyceraldehyde-3-phosphate dehydrogenase (NADP) (phosphorylating) (EC 1.2.1.13) A - rice (fragment	0.7251
P93608_WHEAT	Cold acclimation protein WCOR410c.- Triticum aestivum (Wheat).	0.6684
Q8H4J8_ORYSA	Aldo/keto reductase family-like protein.- Oryza sativa (japonica cultivar-group).	0.5641
Q8H764_WHEAT	Zeaxanthin epoxidase (Fragment).- Triticum aestivum (Wheat).	0.5149
Q9SDV6_WHEAT	Cold-responsive protein COR14a (WCOR14a).- Triticum aestivum (Wheat).	0.3455
Q06540_WHEAT	Cold acclimation protein WCS19.- Triticum aestivum (Wheat).	0.34
T06812	cold acclimation protein WCOR615 - wheat	0.2633
Q8S387_WHEAT	WCOR14c - Triticum aestivum (Wheat).	0.2081
Q9ATG4_WHEAT	Lipid transfer protein precursor - Triticum aestivum (Wheat).	0.108

Three sample replicates from control and cold-challenged plants across a 5-point time series have been prepared by the Quebec group. Nuclear and plasma membrane fractions were purified but only nuclear proteins were sent for ICAT-MUDPIT analyses. Partial datasets have returned and have been examined. A technical problem has been encountered with the protein samples. As an alternative to the facilities at UVic, samples were sent for identical analyses to the Keck proteomic facility at Yale University. Although the ICAT labelling and chromatography steps were as expected, the MS data again did not reveal many sample pairs between the control and cold-acclimated samples. At this point, we do not know the reason for this even though it might indicate some yet unknown protein modification occurring in the nuclei upon LT exposure. Nevertheless, it was decided to suspend the organelle proteomics project and focus on small scale proteomics projects. The first of these is to use biochemical approaches to identify proteins interacting with select wheat CBF transcription factors and other well-characterized cold-induced proteins (TaADF, WCS120, WCOR410, TaIRI). Recombinant proteins have been produced in large quantities and will be used to generate affinity chromatography media. The TaADF-specific column is ready and plant protein extracts will be run through the columns and interacting proteins will be identified by MS analyses performed at the McGill and Genome Quebec Innovation Centre in Montreal. This approach should prove valuable since the yeast two-hybrid assay cannot be used because CBF proteins were found to activate the GAL promoter by themselves. The WCS120 protein will be used to immunize a rabbit for the generation of a new batch of antiserum. This is important since this is the diagnostic tool we use to assess the quality of the protein extracts prepared from cold-acclimated wheat plants. Antibodies will also be raised against the TaIRI-1 protein.

Yeast 2-hybrid cDNA library: The interactomes (interaction map) of TaVRT-1, TaVRT-2 and TaTIL are complete and a manuscript has been accepted by J.F. Laliberté and F. Sarhan groups on the TaVRT1 and TaVRT-2 interactomes. A large number of strong interactors have been identified for a cold acclimation induced receptor kinase F29; further characterization of the positive clones is underway. Interaction screening will be gradually phased out, and emphasis will be put on confirming by BiFC that selected interactions are indeed taking place in a plant cell. A manuscript is in preparation on the interactome for proteins involved in cold acclimation.

E. PROMOTER AND TRANSGENIC STUDIES

1. PROMOTER STUDIES

- Carry out promoter analysis for upstream, potential *cis*-regulatory regions for selected LT-regulated genes. Status: **Ongoing**; putative promoters had been isolated for 21 different genes: WCS120 (homologues from 8 different cultivars), WCOR19, WCOR413-PM1 (A, B and D genomes), WCOR413-PM2, TaADF (homologues from 8 different cultivars), TaTIL (A, B and D genomes), TaVRT-1 (A, B and D genomes from Norstar; homologues from 1 genome of 7 other cultivars), TaVRT-2 (homologues from 10 different cultivars), WPEAMT, WCOR410, TaCBFa, TaCBFb, PpCor413-1, PpCor413-2, PpCor413-3. Five promoters have been tested in transient assays so far, with only WCS120 and TaADF showing cold inducible activity. Transient expression analysis for selected promoters and their characterization in transgenic studies have been initiated.
- Assessment of selected gene promoters regulated by low temperature for genes with different pattern of expression. Status: Completed; six different promoters from 3 families of genes (g-thionin, g-purothionin and lipid transfer protein promoters (4)) had been isolated and inserted into the LRCWheat3-pev vector and transient expression work has been carried out on 4 promoters. The bioinformatics analysis of the promoters of different transcription factors has been initiated and a few constructs were made despite the delay in the microarray study. However, wheat was not transformed with these constructs due to lack of time when the PDF completed her term in June 2005.

Contributing Groups

- Sarhan / Houde (promoter analysis)
- Laroche /Eudes /Gaudet (promoter analysis)

Remarks and Highlights

The analysis of 5 promoters (1g-purothionin and 4 nsLTPs) using an intact wheat leaf transient expression assay and the ability to activate the reporter GFP gene following stimulation by ABA, low temperature fungal extracts and by salicylic acid (SA) and methyljasmonate (MeJA) have been completed by the Lethbridge group. Data has been analysed and presented in different meetings. Some experiments are being repeated and completed. Promoters have been isolated for four new candidate genes using 5'RACE PCR and Genome Walking. For a fifth gene, a large intron was hit which has delayed the identification of the promoter. The WCS120, TaVRT-1, and VRN2 promoters are being used to study allelic variations by the Montreal group. For the WCS120 promoters, no polymorphism was observed on 850 bp fragments isolated from 8 different winter and spring genotypes. In contrast, significant polymorphism was observed between the TaVRT-1 promoters. The three vrn2 copies (A, B, D) have been isolated from cv Norstar and expression profiling for two copies were completed using RT-PCR and is underway for the third. The analysis of the TaADF and WCOR410 promoters is ongoing. Promoters showing good LT inducibility in transient assays will be fused to select genes and transformed to *Arabidopsis* to: a) better characterize their activity and b) evaluate their potential use in biotechnological improvement of freezing tolerance by the Quebec group. The AtTIL promoter fused with the GUS reporter gene was introduced in *Arabidopsis* and its pattern of expression (localization and response to various stresses) is being characterized. Microarray experiments with AtTIL KO *Arabidopsis* lines is ongoing to identify the genes under its influence. The COR413 promoter homologues from *Physcomitrella patens* (PpCor413-1, PpCor413-2, PpCor413-3) were isolated for molecular evolution analyses. In addition, we are isolating the A, B and D copies of TaCOR413-PM2 and TaCOR413-TM1 promoter regions. Transient assays using the WCS120 promoter revealed that cold inducibility is similar at different periods of cold acclimation in both spring (Bounty) and winter (Clair) cultivars. Hence, there is no evidence of deficiency in signal transduction or transcription factor activation that could explain the differential accumulation of this transcript between cultivars during cold acclimation. In total, we have isolated 51 putative promoter regions.

2. TRANSGENIC STUDIES

Objectives

- Promoters. Promoters showing good LT inducibility in transient assays will be fused to various genes and transformed in wheat and *Arabidopsis* to a) better characterize their activity and b) evaluate their potential use in biotechnological improvement of freezing tolerance. Status: **Ongoing**.
- Genes of interest. a) *Arabidopsis* transformation: Gene knockout/RNAi and over-expression of wheat genes. Status: **Ongoing**. Over-expression of CBF1, TaVRT2 , and VRN2 genes under the control of 35S promoter was performed and transgenic plants were produced. The molecular analysis of CBF1, and TaVRT2 transgenic plants are in progress. The bHLH ICE genes were cloned in the binary vector and will be over-expressed. b) Cereal transformation: The project currently underway has three sub-components, i) co-transformation of marker genes and genes of interest for stable expression in wheat, barley and oat cell suspension cultures, ii) optimization of genetic transformation protocols for winter wheat cultivars exhibiting different levels of cold tolerance, and iii) expression of select genes in spring and winter wheat cultivars. Status: **Ongoing**.
- Expression of genes affecting plant carbohydrate metabolism, plant signalling and transcription factors: Status: **Completed;** Plants expressing two different genes have been obtained. The following genes: g-thionin, g-purothionin, lipid transfer protein, three putative transcription factors and a small RNA helicase, were inserted in a new transformation vector. Studies on the impact of expressed transgenes on the transcri ptome are now possible with the availability of the FGAS 17.3 k array.

Contributing Groups

- Sarhan (molecular and physiological analyses of *Arabidopsis* transformants)
- Laroche (with Eudes/Gaudet/Graf, transformation protocols, selection strategies, phenotypic evaluation)
- Parkin (Gene knockout/RNAi and over-expression of wheat genes in *Arabidopsis*)
- **Fowler** (Phenotypic characterization and LT_{50} determinations)

Remarks and Highlights

Arabidopsis

Single and double insertions homozygous lines over-expressing TaTIL-1, TaTIL-2, AtTIL, AtCHL, TaCHL, WPEAMT, AtTILprom::GUS, AtCHLprom::GUS have been isolated and seed stock are being collected. Knockout (KO) lines for AtADF, two AtCOR413 homologues, two AtPEAMT homologues, four AtTIL, and one AtCHL have also been identified from the SALK collection. Homozygous lines are being selected for phenotype analyses. The progeny will be tested for FT through replicated electrolyte leakage and re-growth tests. Re-growth tests with one of the four AtTIL KO lines demonstrate that non-acclimated KO plants have a 40 % higher mortality rate than non-acclimate wild-type plants when frozen at temperature of -4 to -6 °C. RNAi constructs were also generated for AtCOR413-PM, AtCOR413-TM , and AtTIL, lines carrying single insertions have been identified and have been grown out to confirm silencing. No silencing was observed with AtTIL RNAi lines.

Wheat.

Spring and winter wheat cultivars have been transformed with TaVRT-1 and TaCBF9 in Chibbar's laboratory to assess their role in controlling downstream gene expression involved in cold tolerance and vernalization. Both genes were inserted in the sense and anti-sense orientations downstream of a constitutive promoter (rice actin1-D). The transgenic plants have been advanced to the next generation. The status about the seeds and the characterization of these plants using biochemical and molecular techniques are uncertain as this part of the program was dropped as per recommendations of the steering committee (December 2003).

Plants expressing two different genes SFT (sucrose fructosyl transferase, a gene involved in fructan accumulation) and NahG (a gene preventing the accumulation of salicylic acid thereby disturbing signal transduction) have been regenerated, selected and are being characterized by the Lethbridge group. These plants should provide information on how constitutive expression of these genes affects plant response to abiotic stress and how presence of these genes affects expression under control and low temperature environments. Their expression vectors have been improved to include adapters for directional cloning, a new antibiotic resistance gene and lethal genes to increase selectivity to identify new constructs. Five genes (3 nsLTPs, 1 g-thionin, 1 g-purothionin) under the control of the rice actin constitutive promoter have been bombarded on immature wheat scutella. Plantlets have been obtained and are completing their life cycle. A salt-rich medium assay has been developed to identify plants expressing the NahG gene. New PCR primers were generated to facilitate the identification of these transgenic plants. Additional NaGh seedlings subjected to different low temperature treatments are being grown for RNA extraction and microarray experiments. A few lines originating from five independent events and displaying a very strong tolerance to salt have been identified and the presence of the insert has been verified in over 75 derived lines using an improved PCR protocol. Plants are being subjected to various stress treatments. Shifts in the transcriptome have been probed with the FGAS wheat array and differences were observed within cultivars as reported above. The protocol for identification of the SFT transgenic plants is being reviewed and new promoters have been designed to facilitate the identification of these transformed lines. New genes have been inserted in the stable transformation vector pLRC gev. T1 seed have been obtained for 2 transformed genes in the spring genotype and in the winter wheat cultivar Jagger. T1seeds for 4 genes in the Norstar cultivar will be available shortly. T1 seeds are being advanced to the next generation. RT-PCR is being used to characterize the different lines obtained. No abnormal phenotype was observed in all these transgenic plants. We have continued to replicate the work with 5 different promoters and deletion series into intact leaves of spring and winter wheat cultivars. Seeds from the different lines have been harvested (almost completed as seeds from only a few plants as they mature are left to be collected) and being stored until they will be analysed under a different project. A limited number of lines are being analysed under a different project now.

II. BRASSICA STRESSES THEME

The *Brassica* component of this GP project utilizes a wide range of genomic technologies to study the response of *Brassica* species and the related model species *Arabidopsis thaliana*, to abiotic stress. As a result of a recent refocusing exercise, the team is committed to examining low temperature, drought and nutrient stress with an increased emphasis on investigating lead candidate genes. Comparison of differential expression profiles are assisting in uncovering both the unique and specific roles that sets of genes play in determining a plant's ability to survive different abiotic stresses. The *Brassica* team has made substantial advances in meeting the goals of the project, which has involved establishing growth conditions, setting up experimental protocols and the accumulation of gene expression data at both the transcript and protein level. A project website has been set up to allow data to be deposited; this will allow genes/expression patterns common to each of the stresses to be identified. This final year of the project will require some bioinformatics based analysis to allow interpretation of the amassed expression data, and to define commonalities and differences between the stresses in both *Brassica* and cereals. The focus of the group will be on the characterization of novel *Arabidopsis* genes and their *Brassica* homologues. Below, progress is presented under each of the 4 research themes in relation to the original objectives. The percent completed indicates the progress towards the end of project (March 2006) milestones (see Gantt chart in Appendix for tracking of milestones).

A. IDENTIFICATION OF NOVEL GENES CONTROLLING FREEZING AND DROUGHT TOLERANCE IN *BRASSICA/ARABIDOPSIS*

Overall Goals

- To identify novel genes controlling freezing tolerance in B. napus and Arabidopsis and to investigate the role of select genes in conferring cold tolerance in Arabidopsis and B. napus.
- To investigate the role of abscisic acid (ABA) in regulating pathways involved in cold and drought tolerance.

Contributing Groups

- **Gusta** (cold acclimation, proteomics)
- Parkin (cold acclimation, SAGE analysis, microarray analysis, gene characterization)
- **Cutler** (drought, ABA response, microarray) (Outlined under Theme IV)
- Muench (cold acclimation, protein phosphorylation)

Objectives and Outcomes

1. Construct serial analysis of gene expression (SAGE) libraries from Arabidopsis tissues subjected to a time course of cold stress. **Status: 100% Complete. Six SAGE libraries have been constructed.**

2. Conduct sequencing in the described libraries, to generate at least 250,000 quality SAGE tags from the *Arabidopsis* genome. **Status: 100% Complete**. This objective has been exceeded, improved library construction techniques and cheaper more efficient sequencing protocols allowed almost 500,000 tags to be identified. A manuscript describing the analysis of five SAGE libraries is almost complete and will be submitted before the end of the year.

3. Comparative microarray analysis for three crucifer species of varying freezing tolerance: *B. napus*, *A. thaliana* and *Thlaspi arvense* using *Arabidopsis* oligo arrays. **Status: 100% Complete.** Gene expression was analysed in *T. arvense* utilising Arabidopsis microarrays, this data has been compared to publicly available Arabidopsis mciroarray data. Over 700 genes with differential expression were identified; expression patterns have been confirmed using RT-PCR in Arabidopsis and *T. arvense* for a number of these for publication purposes. Further analysis based on gene annotation has identified candidate genes for further work and constructs to characterise two of the genes are being developed. . **Objective (March 2005):** Characterise the role of two candidate genes identified through the expression profiling. Homozygous T-DNA knock-out lines have been identified for two candidate signalling proteins. Phenotypic characterisation has been completed for one line and analysis of the second KO line is on-going. Lines over-expressing each of the candidate genes have been generated, the T2 generation is presently being grown out.

4. Comparative bioinformatic analysis of gene expression in *A. thaliana* in response to cold, drought and ABA (see Theme IV). **Objective (March 2005):** Complete analysis. **Status:** The majority of the

microarray data has now been collected for each stress and deposited at the project website. We will shortly hold joint meetings among the PIs who generated the data to identify the most suitable analyses to proceed with this objective. A bioinformatics summer student was hired to expedite this process and the data from the different experiments is being collated along with available data from the wheat group.

5. Towards functional characterisation of five candidate genes. **Status: Ahead.** *Arabidopsis* T-DNA knockout lines have been identified for 8 candidate genes and over-expressing lines have been developed for eight of these same genes. **Objective (March 2006):** Complete characterisation of gene KO lines and over-expressing lines. **Status: 40% complete.** LT50 tests using electrolyte leakage have been performed on three of the KO lines and three of the over-expressing lines. An LT50 test by whole plant re-growth was carried out for one additional over-expressing line. We had some delays in phenotype analysis in the last quarter due to equipment failures which unfortunately required us to replace some essential pieces of equipment. Whole plant freeze tests are almost complete for these lines and replicated experiments will have been completed by the end of August, 2005.

6. Use MALDI TOF MS and Q/TOF-MS/MS to identify 2D PAGE separated proteins that contribute to the cold acclimation process in *B. napus*. **Status: 100% Completed** for 2D-PAGE identification of proteins, **100% Completed** for MS analysis of identified proteins, and **70% Complete** for characterization of proteins and determination of function **Objective (March 2006)**: Over the past three months we have been working on collaboration with GE Healthcare conducting a complete DIGE analysis of our samples. We are in the process of utilizing MDLC MS/MS to identify the proteins and hope to have an additional 100 proteins identified.

7. Establish phosphoprotein profiles and identify proteins that show differential phosphorylation patterns under cold and ABA treatment. **Status: 95 % Complete.** Robust protocols have been established. **Objective (March 2006):** Several putative candidate proteins have now been identified that are phosphorylated in response to low temperature using Pro-Q Diamond phosphoprotein stain as well as in vivo labeling using 32P. We have now completed this analysis for short term treatments (up to 30 minutes) using temperatures of 4C and 12C and are in the process of purifying these candidate phosphoproteins. Approximately 10 proteins are preferentially over or under phosphorylated under these conditions. Replicated ABA treatment experiments are near completion, and several candidate proteins have been identified that change in their phosphostatus using 32P pulsed labeling. We have identified several proteins by spot excision and LC-MS/MS. Two phosphoproteins that respond to 2 min of low temperature stress are of interest and will be characterized further. These include a dehydrin and a nucleotide diphosphate kinase. We are now using immuniprecipitation methods to confirm the short-term phosphorylation of these proteins.

8. Large-scale identification of microtubule-associated proteins from *Arabidopsis*. **Status: Completed**. 130 proteins unambiguously identified, manuscript published. **Objective (March 2006):** Identification of microtubule-associated signaling proteins that are phosphorylated or released from microtubules in response to cold stress and ABA treatment. Protein profile comparisons between control and treated plants are now underway. **Status: 45% complete.**

Remarks and Highlights

Six SAGE libraries have been constructed from leaf tissue of: plants grown under normal conditions, plants transferred to 3°C for 30 min, 2 hours, 48 hours and 7 days. A second control library was also constructed to assess the reproducibility of the technique. Almost 500,000 SAGE tags have been extracted from these libraries and these data are presently being analysed to provide a comprehensive transcript profile during cold acclimation for Arabidopsis. These data are forming the basis for a manuscript which will be completed and submitted shortly. Functional characterization is already underway for candidate genes, homozygous *Arabidopsis* T-DNA knockout (KO) lines have been identified for 8 candidates (7 from the SALK collection (http://signal.salk.edu/cgi-bin/tdnaexpress) and one from AAFC's in-house collection). These lines are presently being phenotypically being tested using electrolyte leakage and re-growth assays to assess the role the gene plays in cold acclimation, three lines have now been tested. Over-expression constructs have been generated for 8 of the same genes and transformed into *Arabidopsis*. Primary transformants have been identified. Homozygous lines have been identified for six of the over-expressing constructs, we are presently confirming copy number of the inserts, and the expression level of the inserted genes has been confirmed for each of the transgenic lines. Whole plant freezing tests are presently being conducted for each of the lines. LT50 tests have been carried out for four over-expressing lines, one of the lines appears to be showing a positive increase in freezing tolerance

compared to wild type but further confirmation will be necessary. There has been a slight delay in phenotype analysis due to equipment failures which necessitated the purchase of essential equipment to complete the freezing studies. However, one of the lines over-expressing a potential signalling protein is conferring a surprising but interesting phenotype which relates to water loss. In response to a shift from high to low humidity the over-expressing line wilts dramatically compared to wild type suggesting the closure of the stomata is being impaired.

Molecular genetic investigations are also underway using low temperature tolerant *T. aravense*. The microarray hybridisations have been completed for the *T. arvense* RNA, the data analysis has identified over 700 differentially expressed genes. These data has been compared to publicly available expression data for Arabidopsis subjected to cold, drought and ABA treatment. The manuscript describing these data will be submitted before the end of 2005. Two genes, one putative signalling molecule and a gene involved in the oxidative stress response have been selected for further characterisation; they are presently being cloned into over-expression constructs for phenotype studies. Homozygous T-DNA knock-out lines have been identified for two candidate signalling molecules. Self seed of these lines has been collected and will be subjected to various abiotic stress conditions. Phenotypic characterisation of the first KO line identified no significant differences in its response stress compared to WT, although it does show a difference in flowering time. The second KO line is in the *Landsberg erecta* background and appears to have lost the classic short stature associated with the *erecta* genotype, tests for its response to stress are presently on-going. The over-expressing lines are at the T2 generation and seed is being collected for phenotypic testing.

We are in the process of preparing five manuscripts, directly related to the funding received from Genome Prairie, on the development of freezing tolerance in *Brassica napus*. In the past quarter we were fortunate enough to have a small "proof of principle" analysis conducted for us by GE Healthcare which used 2D-DIGE to compare proteomes of differentially cold treated canola samples. The analysis identified an additional 33 LT associated proteins which we are currently characterizing. Due to the excellent results obtained in this small study, GE Healthcare has agreed to conduct a full proteome analysis of one of our experiments for presentation at the ASMS meetings in Texas in June of 2005 as well as to use in GE Healthcare marketing publications (no specific information regarding the LT research will be disclosed). In addition to the full scale 2D DIGE analysis we are also continuing characterization work on proteins we previously identified, and are potentially exploring additional funding sources to continue the characterization work on these proteins.

Protein phosphorylation studies have identified at least 10 proteins that are specifically over or under phosphorylated under low temperature stress conditions and are likely candidate signaling proteins that are important in the cold stress response. The first approach exploits the established role of protein phosphorylation in widespread signaling events. We have identified several proteins that change in their phosphorylation status in response to low temperature stress treatment using the Pro Q Diamond (Molecular Probes) phosphoprotein stain and by 32P in vivo labeling. We have successfully repeated these experiments, and are now in the process of purifying these proteins for their subsequent identification. Using 32P labeling, we have identified several proteins from Arabidopsis cell suspensions that are hyper- or hypo-phosphorylated in response to 20 um ABA treatment. We are now preparing a manuscript on these phosphoproteome changes in response to low temperature and ABA treatment using Pro-Q Diamond and 32P labeling as indicators of protein phosphostatus. Numerous signaling proteins have been identified from the 130 proteins that we have sequenced in our microtubule binding protein fraction, and these microtubule binding proteins are being characterized for their roles in cold and ABA induced phosphorylation events.

B. EFFECT OF ABIOTIC STRESS ON GENE EXPRESSION IN MICROSPORE-DERIVED (MD) CULTURES OF *BRASSICA*

Overall Goals

- To identify genes in MD cultures induced/repressed in response to abiotic stress.
- To uncover the role of lipid metabolism in resistance to abiotic stress.

Contributing Groups

- Moloney (MD embryos culture, microarrays, lipid biosynthesis)
- Weselake (proteomics, subtraction libraries, macroarrays/microarrays, lipid biosynthesis)

Objectives and Outcomes

1. To investigate the effect of abiotic stress (low and high temperatures and osmotic stress) on the proteome (using 2D PAGE/mass spectrometry) of microsomal fractions of MD cell suspension cultures. **Status: 95% Complete.** Of the twenty-one proteins that were identified to be differentially expressed in response to either low temperature or osmotic stress, assays were developed for three of the nine enzymes identified: acetolactate synthase (ALS), beta-glucosidase and succinate dehydrogenase.Microsomal fractions from both control and challenged cells were prepared and the enzyme activities were assayed in order to validate 2D-PAGE/MS results. ALS activity from both cold-challenge timepoints supported corresponding spot density data. The investigation of differential microsomal protein expression in response to heat stress (35° C for 2 h) and heat stress accompanied by osmotic stress (35° C and 20% (w/v) PEG 4000 for 2 h) is ongoing. **Objectives (March 2006):** To continue validation studies, using real-time RT-PCR for the candidate genes identified through 2D-PAGE and MS. This approach will also allow us to expand our work to include the investigation of non-enzyme gene products (e.g., actin). Primers and probes for *DGAT1* and *DGAT2* from *Brassica* have recently been obtained and those for other transcripts are being developed. Transcript profiling in response to cold-challenge and osmotic stress will be determined. DGAT activity has been shown by others to increase in response to cold temperature exposure in *Arabidopsis*. Experiments to determine LT_{50} and chromosomal ploidy level of the cell suspension, concerns raised recently by other FGAS researchers, are also in preparative stages

2. To investigate the effect of abiotic stress on transcript levels in MD cell suspension cultures and MD embryos. **Status: 100% Completed**. Suppression subtractive hybridization (SSH) cDNA libraries for 12h (CA12) and 72h (CA72) cold acclimation - low temperature (up-regulation), 12h osmotic stress (PG12, down-regulation) and 72h osmotic stress (PG72, up-regulation) have been completed. Sequenced clones from the CA72 (forward) and PG12 (reverse) SSH libraries gave the following results, reported as the number of unique genes grouped in functional categories (first value is for unique CA72 forward sequences, second, in parentheses, is for PG12 reverse): cytoskeletal components, 13 (0); cell signaling, 9 (1); amino acid and protein metabolism and turnover, 28 (3); polypeptide recognition and folding, 8 (2); cell cycle regulation and chromatin structure, 16 (5); carbon metabolism, electron transport and bioenergetics, 40 (21); protein synthesis, 39 (52); transcription and post-translational modification of mRNA, 29 (11); kinases/phosphatases, 21 (2); ion transport and subcellular trafficking, 38 (6); development, 1 (3); miscellaneous stress-related proteins, 20 (2); unclassified proteins and those with unknown function, 83 (49). Of the 505 *Brassica* genes identified to be associated with either the cold and osmotic stress responses to date, 33 have also been observed in wheat following low temperature exposure in the companion project within FGAS.

Objective (March 2006): Sequencing of SSH libraries from other treatments will continue.. cDNA and SSH libraries will be constructed, giving us a full complement of libraries from all treatments and timepoints. Of the genes identified through SSH, several of the more promising candidates will be selected and real-time PCR probes and primers will be designed and used to validate the roles of these genes in the abitoic stress response.

3. To investigate the effect of low temperature and osmotic stress on gene expression associated lipid metabolism in MD cultures. **Status: 80% Complete**. MD cell suspension cultures were grown under control (25° C) and both abiotic stress (5° C and 20% w/v PEG 4000) for 12 and 72 h. The light membrane fraction (LMF) was prepared from each treatment following (10-45% w/v) sucrose density gradient (SGD) ultracentrifugation. SGD fractions from all treatments were assayed for DGAT activity. As observed in previous experiments, a LMF isolated between 18 and 30% sucrose was enriched in DGAT activity. Initial analysis of the data across the treatments, however, suggested that DGAT activity neither increased nor decreased for either of the two abiotic stress treatments. Additionally, these SGD fractions were subjected to 2D-PAGE and gels subjected to image analysis. For each gel there were five to eight, clearly identifiable, protein spots. These spots will be excised and analyzed by mass spectrometry.

Objective (March 2006): With respect to micro-purification of type-1 DGAT polypeptide, tissue culture material was prepared and a LMF was isolated following SDG ultracentrifugation. Electrophoresis and identification of DGAT spots using anti-DGAT antibodies in Western blotting experiments combined with picking putative DGAT spots from the gel and performing fragmentation and mass spectrometry analysis will be completed once the appropriate instrumentation has been obtained or located.

4. Complete microarray analysis to identify critical cDNAs. Transform Arabidopsis with critical cDNAs (knockout/over-expression) and assessment of phenotypes. **Objective. 75% Complete.**

The effect of over-expressing transcription factors ABI3, ABI5 and 14-3-3 on gene expression and responses to cold acclimation were examined in *Arabidopsis*. Gene expression patterns in *Arabidopsis* plants overexpressing the ABI3 gene were compared with that of wild type (i.e. ecotype C24) using the Affymetrix microarray platform. Overall, the microarray approach identified numerous differentially expressed genes (i.e. up- and down-regulated genes) sensitive to ABI3 expression. These included a significant number of genes encoding transcription factors or lipid metabolism enzymes. Given that over-expression of ABI3 imparts enhanced cold tolerance in *Arabidopsis*, some of the genes identified may be responsible for the stress-resistant genotype.

Currently, triple-, double- and single-gene transformants of the three transcription factors (i.e. ABI3, ABI5 and 14-3-3 protein) have been produced Currently, triple-, double- and single-gene transformants of the three transcription factors (i.e. ABI3, ABI5 and 14-3-3 protein) has been produced using successive transformations or super-constucts. The expression of these genes were driven by a constitutive promoter (i.e. ubitiquin) Large phenotypic differences were observed in the transformants, particularly with ABI3 plants and the triple-gene transformants. Preliminary experiments of cold (i.e. culture of plants at 4 °C for a 6 weeks) and freezing (i.e. temperatures to a minimum of -14 °C) stress revealed that the double transformants (i.e. ABI3 and ABI5 genotypes or ABI3 and 14-3-3 genotypes) had higher growth rate compared with single-gene transformants and wild type genotypes at low temperatures. The double transformants were also less injured in their vegetative part and had significantly better development of siliques at freezing temperatures. A formal experiment is ongoing to assess via gene segregation a possible correlation between the responses of cold and freezing tolerance and particular phenotypic characteristics observed in the transformants (i.e. length of internode, content of chlorophyll, etc) and hormonal profiles.

The three transcriptions factors are being used for *Agrobacterium* transformation of *Brassica napus* using cotyledon as explants. Single-, double- and triple-gene transformants has been obtained by using various selectable markers. The confirmation of the presence of the ABI5 gene on the transformants was carried out by PCR. The detection of the other genes (i.e. ABI3 and 14-3-3 genes) is pending.

A study designated to assess protein-protein interactions and the DNA binding characteristics of the three transcription factors is underway as well. An initial step was the production of their antibodies. The ABI5 and 14-3-3 genes were successfully expressed in *E. coli* using the vector pRSET C, as confirmed by results of protein sequence provided by the laboratory facilities of the University of British Columbia. Both proteins were purified by affinity chromatography and injected into rabbits for further production of their antibodies in Prof. Moorhead's laboratory at University of Calgary. The production of ABI3 protein in *E. coli* was not successful so that a short peptide (i.e. nine amino acids) of its C-terminal was chemically synthesised. This ABI3 peptide was coupled to bovine serum albumin to be used as antigen to attempt the synthesis of ABI3 antibody. Strong detection signals were observed in western blot using sera from rabbits injected with ABI5 and 14-3-3 proteins when tested with purified proteins and *Arabidopsis* crude extracts. The assessment of the performance of the ABI3 antibody is pending. The study of protein-protein interactions between the three transcription factors will be carried out by immunoprecipitation. Furthermore, a potential transcription factor (i.e. a protein with potential DNA binding capacity) upregulated by ABI3 is being isolated by RT-PCR to assess its possible interaction with the other three transcription factors.

To study the DNA binding properties of these three transcription factors, potential promoters of the following genes involved in lipid biosynthesis and ABA signalling are being isolated to be assessed via gel retardation: chloroplast glycerol-3-phosphate acyltransferase (G3PAT), 3-ketoacyl-ACP synthase II (KAS II) and Δ9-stearoyl-ACP desaturase (SD), acetyl-CoA carboxylase (ACCase), phospholipase C and D.

The DNA sequence of the coding region of ABI3 showed the presence of a stable hairpin at high temperatures (i.e. >90 °C). A study is being conducted to assess the effects of a ABI3 sequence without hairpin. The ABI3 potential promoter (i.e. DNA fragment of 1700 bp) and terminator (i.e. DNA fragment of 750 bp) were isolated using PCR and ligated to the coding region without hairpin as well as the wild type sequence (i.e. ABI3 sequence with hairpin). Both gene cassettes are planned to be inserted into ABI3 knockout plants obtained from the Arabidopsis Biological Resource Center. A parallel experiment which studied the phenotypic effects of the over-expression of the ABI3 sequence without hairpin in *Arabidopsis* plants showed that this DNA sequence produced a decrease in the vigor of plants, especially

in transgenic plants where ABI5 and 14-3-3 genes were over-expressed. Additionally, the cloning of a potential *Arabidopsis* transcription factor (i.e. a zinc finger protein) highly up-regulated by ABI3 was undertaken. This transcription factor has potential RNA binding properties which may increase the stability of ABI3 transcripts.

As chloroplast desaturation may be an important mechanism of stress resistance, a study using the G3PAT, KAS II, SD and ACCase genes encoding lipid enzymes was undertaken to assess their effects. The first three genes were used for transformation of *Arabidopsis* plants in a constitutive manner. Subsequently, triple-, double and single-gene transformants were obtained. Surprisingly, transformants with chloroplast G3PAT and KAS II showed reduced growth and seed development. The negative effects of these genes were reversed with the insertion of the Δ9-stearoyl-ACP desaturase into the plants. The forth gene (i.e. acetyl-CoA carboxylase) was isolated by RT-PCR and recombinant PCR and, subsequently, sequenced. An assessment of the plant responses of the transformants with lipid enzymes to low temperatures was carried out. The single-gene transformant containing either KAS II or SD showed the highest growth and development in spite of their lower performance in normal growth conditions. An experiment to assess the tolerance of these genotypes to freezing temperatures is in preparation.

Besides the transcription factors ABI3, ABI5 and 14-3-3 protein, candidate genes were selected for *Brassica napus* utilizing the information produced from microarray analysis of *Arabidopsis* plants over-expressing ABI3. A *B. napus* homologue of a potential *Arabidopsis* transcription factor (i.e. a zinc finger protein) was cloned and transformants of canola containing this gene have been produced.

Remarks and Highlights

Our results in this research project have shown that the over-expression of transcription factors (i.e. ABI3, ABI5 and 14-3-3) involved in early events of the ABA transduction pathway had a significant enhancement on cold and freeze tolerance of *Arabidopsis* plants. Many metabolic pathways particularly hormone and lipid biosynthesis were affected, according to early microarray evidence. An interesting question is how changes in lipid composition due to alterations in lipid biosynthesis are modulated by transcription factors combined with ABA turnover. In this respect, an extension of our research scope was necessary. Our studies of transformants containing the three transcription factors and transformants with altered lipid biosynthesis, typically consisted of functional genomic techniques, have now been extended to include biochemical and physiological components. Consequently, a parallel study on hormone profile, focusing in ABA metabolites, and lipid analysis is in preparation using chromatographic techniques involving Drs Abrams, Pharis and Weselake. The measurement of physiological variables, such as electrolyte leakage, photosynthetic efficient and content of pigments, will be carried out to understand better the involved mechanisms. Growth chamber with a precise control of temperature, provided by Prof. Gusta of the University of Saskatchewan, will be used for our plant freeze tolerance experiments.

Initiatives were undertaken to gain a deeper insight into the mechanisms by which the transcription factors regulate gene expression by studying their protein-protein interactions and transcriptional regulation. Using the facilities of Dr Moorhead's lab at University of Calgary, the production of antibodies, which are key components for protein interaction studies, are producing promising results. The alteration of the DNA sequence of ABI3 generated interesting phenotypic changes in the *Arabidopsis* plants. Further studies with RNA binding protein (i.e. zinc finger protein) may provide better understanding of the mechanisms that ABI3 uses to confer tolerance to cold stress. Additionally, the cellular localisation *in vivo* of these transcription factors is ongoing through fusion with fluorescent proteins and visualisation by laser confocal microscopy.

C. PROTEOMIC AND GENOMIC ANALYSIS OF ABIOTIC STRESSES IN ROOTS

Overall Goals

- To identify novel genes in *B. napus* and *Arabidopsis* associated with specific environmental stresses including nutrient stress.
- To target signaling molecules through proteomics.
- To identify all proteins exuded by canola roots under different environmental stresses.
- To study the role of primary metabolism enzymes in root responses to Pi and cold stress.
- To identify gene expression changes associated with phosphate stress.

Contributing Groups

- Taylor (Root exudate proteome, aluminium and nutrient stress)
- Good (Nutrient stress, genetic resources)
- Rivoal (glycolytic enzyme interactions under stress)
- Hill (hypoxic stress) - milestone was deleted in accordance with the Site Visit Committee recommendations
- Muench (MAP analysis, phosphoprotein analysis, aluminium stress)

Collaborating Groups

- Deyholos (microarray analysis)

Objectives and Outcomes

1. Characterize the microtubule associated proteome (MAP) of Arabidopsis (estimated to be approx 100). **Status: Completed.** 85 protein plugs were sent for MS analysis, 150 proteins were unambiguously identified.

2. Establish phosphoprotein profiles for stressed tissues and characterize changes. Status: 50 % Complete. Robust protocols have been established. Objective (March 2005): Study changes in phosphoprotein profiles in Arabidopsis under Aluminium (Al) stress.

3. To generate an Extracellular Plant Proteins database (EPPdb). **Status: Completed.** Isolation of low abundant proteins from root exudates has been optimized. From an estimated proteome size of 60-70 proteins, 16 proteins have been identified through MS analysis. A preliminary Extracellular Plant Proteins Database has been set up (http://www.cs.ualberta.ca/~wyang/EPPdb/; Wang et al., 2004). A program is being created to predict putative extracellular proteins using amino acid sequences. We have continued the Bioinformatic analysis of identified extracellular proteins by determining the signal peptides and the presence/absence of transmembrane domains. Based on their functional classification the extracellular proteins belong to a number of different gene families including peroxidases, blue copper binding proteins, trypsin and protease inhibitors, glycoside hydrolases and superoxide dismutases. Root exudate proteins were collected from *Arabidopsis* and sent for analysis by Multidimensional Protein Identification Technology (MudPIT). A total of 52 proteins were identified by the MudPIT analysis. These *Arabidopsis* proteins have been analysed to determine their functional classification and the presence/absence of signal peptides and transmembrane domains. A single manuscript describing the *Brassica* and *Arabidopsis* extracellular proteins is now in the final stages of preparation for submission to Plant and Cell Physiology.

4. Identification of proteins exuded under different stress conditions. **Status:** 75%. Aluminum stress conditions have been optimized. Due to a decrease in the amount of extracellular proteins exuded under Al stress conditions, additional collection and concentration was required. We now have completed the collection and processing of enough exudates from control and Al-stressed plants to begin 2D analysis. This processing involved concentration and desalting using a pressure ultrafiltration system (Amicon 8200) with YM3 membrane, followed by a final concentration using a Speed Vac concentrator. The small-scale sterile system to study phosphate (Pi) deficiency was optimized using a modified pretreatment system and a more frequent transfer protocol. Solution acid phosphatase activity is being used as a marker of the deficiency in Pi deficient plants. We have also completed the collection and processing of all of the exudates under control and Pi deficient conditions and have begun preparation for 2D analysis

We are also investigating the use of a *B. napus* cell suspension culture as an alternative method for examining extracellular proteins exuded under stress conditions. Preliminary experiments suggest that this culture responds to phosphate stress in a similar manner to our plant/vessel system (increase in solution acid phosphatase activity, decreased culture growth). In addition, the cell culture generates significantly more protein than the vessel system. This allows for collection of enough protein for 2D analysis from a single experimental run as opposed to the multiple experiments needed from the vessel system. The increase in protein allows us to employ more efficient collection techniques, further maximizing protein recovery during concentration. The results of the 2D analysis from the vessel experiments will provide an important reference to which the cell suspension culture results can be compared.

5. Establish growth conditions for quantifying Pi and nitrogen (N) deficiency, and study associated changes in gene expression. **Status: Completed.** We have investigated the genetic response of the crop plant canola (*Brassica napus* L. cv Westar) to Pi stress. Using a hydroponics system, plants were allowed to deplete Pi from solution and were then maintained in deficiency until visual symptoms were observed

(presence of anthocyanins in leaves). At this point, the plants were returned to control Pi levels and allowed to recover. Changes in gene expression throughout all stages of Pi stress were examined by comparing RNA extracted from both stressed and control plants using microarray analysis. We have completed this comparison in root and shoot tissues and are analyzing the data in order to 1) identify novel genes involved in the perception, signal transduction, and response to Pi stress and 2) understand the coordinated regulation of cellular metabolism in response to the perceived stress. This analysis has indicated that there are distinct subsets of genes that are differentially expressed in root and shoot tissues. There is also a set of genes that are differentially expressed in both tissues. Not surprisingly, a larger number of genes are differentially expressed in the shoots compared to the roots. Although work studying changes in specific genes during Pi stress has been published, our initial work has demonstrated that *Arabidopsis* chips can be used to monitor changes in gene expression in *Brassica* and we have identified changes in gene expression which were not previously observed in *Arabidopsis*. This work was presented at the FGAS meeting and Plant Canada 2005 (Edmonton) and is being written up. (Bryman et al. 2005)

We have overlain the observed expression profiles on a metabolic map which has allowed us to make predictive hypotheses regarding the induction of gene expression in *B. napus* under Pi stress. Genetic resources, including both inter and intra-specific crosses within the *Brassica*, have been developed for studying nitrogen use efficiency (NUE) and are being analysed in the field. We have phenotyped a number of our parental genotypes in two different field seasons and have developed mapping populations which should be segregating for NUE. These populations have been used to generate a genetic map using RFLP markers. We have completed two seasons of initial field trial characterization of these lines. This work has been completed by a Ph.D. student in my lab, (Terry James). Initially (Summer, 2003) nine genotypes of *Brassica* napus and 6 rates of applied nitrogen in the form of urea were used in a split plot design. The nitrogen treatments are the main treatment and the genotypes are the sub-plot. Because of the drought of 2002 most soils around Edmonton had high levels of nitrogen and so the trial was seeded at a location near Dundurn, Saskatchewan in May, 2003. During 2003, early season dryness and heavy flea beetle and grasshopper infestations limited emergence in some plots, however we were able to collect field trial data. The data collected has been statistically analyzed for 2003 and some general trends were observable. There is a general trend for delayed flowering in most lines with increasing nitrogen rate. There is also a general increase in plant vigor in most lines with increasing nitrogen. The vigor rating recorded was a visual rating on the appearance of the plants taking into account height, greenness of the foliage, and degree of branching. These trends are most notable in the lines Maluka and LKO-569. They may indicate an ability of these lines to make greater use of applied nitrogen. Additionally, the lines tested showed variability in emergence under stress conditions, a trait we may also be able to map.

In 2004 the trial was repeated at Ellerslie and Vegreville. The responses of the line in terms of plant growth in response to nitrogen were obvious in these trials and were similar to last year. We have also chosen a mapping population, based on our 2003 research and are completing seed increases and an initial NUE evaluation this summer. The mapping population we have chosen segregates for a large number of characters including days to flower, days to harvest, plant height, plant biomass, pod size, seed size, and of course seed yield. Because seed of this population was limited we were only able to conduct single row comparisons in 2004. However seed from all lines will be available for field work in 2005. Frome the single row comparison we should be able to make some some preliminary observations on seed yield/unit N applied as well as protein content and N harvest index. We intend to conduct more intensive phenotypic analysis on lines that show extensive differences, including leaf area measurements, growth rate analysis and N leaf content. This observations may be helpful in helping calibrate fertilizer recommendations. Currently leaf analysis is occassionally used to make commendations regarding the need for additional nitrogen application but more work would be helpful to improve the reliabilty of these tests. We have also observed that some lines seem to show signs of nitrogen stress earlier than others. With the funding provided we will be able to continue towards understanding this trait.

Objectives (March 2006): Completed confirmation of *Brassica* differentially expressed genes as homologues of those on the *Arabidopsis* microarray. **Status:** 70%. With the completion of the shoot microarrays, we have begun a global examination of all differentially expressed genes for identification of candidate genes for future study. Optimization of real-time PCR for secondary confirmation of differentially expressed genes is now continuing on a gene by gene basis. Prior to proceeding with secondary confirmation, the identity of a subset of *Brassica* differentially expressed genes will be

confirmed by a PCR approach. We have now confirmed the identity of 4 genes from the microarray analysis with previously identified homologues in *Brassica*. Real time confirmation of expression patterns is now underway for these genes. In cases where a *Brassica* homologue of the identified gene is not available, primers will be created using the *Arabidopsis* sequence, including one developed from the70-mer sequence spotted on the microarray, and RT-PCR products obtained from these primers and *Brassica* RNA samples will be sequenced. This strategy has yielded single RT-PCR products of expected size for an additional 6 genes. We are currently sequencing them to confirm that we have identified *Brassica* homologues of the *Arabidopsis* genes present on the microarray. Real time confirmation of expression patterns will follow shortly. To assist in characterizing candidate genes, protocols are being optimized to express His-tagged proteins in *Arabidopsis* roots and isolate His-tagged proteins from transgenic roots and a steady-state phosphorylation assay is being established, which complements the pulse phospholabeling experiments.

6. To affinity-purify interacting protein partners for 26 proteins/enzymes of primary metabolism *in planta*. Isolation and sequencing of full-length cDNA clones **completed**. Cloning into the his-tagging bacterial vector pProHex HT **completed.** Specific polyclonal antisera have been produced against 70% of target enzymes. 65% have been transferred into *Arabidopsis* using *A. rhizogenes*. We are overexpressing enzymes *in planta* (roots) to fish out their interactors. Specificity of the interactions is checked by (i) comparing extracts of His-tagged overexpressors to extracts of *A. thaliana* roots transformed with the empty vector and (ii) analyzing extracts by Fast Protein Liquid Chromatography-Immobilized Metal Affinity Chromatography (FPLC-IMAC) (0.8 mL Ni^{2+} column eluted with a 20-250 mM immidazole gradient). Over expression of His-tagged versions of primary metabolism target genes has been achieved with a success rate of 65%. We had little or no significant overexpression for the rest of the target genes based on immunoblot screening or activity assays. They have therefore been set aside as a low priority. Three interactors have been observed with His-NDPK (nucleoside diphosphate kinase) and 2 other with His-HK2 (a cold-inducible hexokinase in *A. thaliana*). One of these interactants has been identified as Snf1, a protein kinase involved in sugar signaling. His-HK2 is partially associated with a high molecular weight complex (>400 kDa) containing protein kinase activity in transgenic roots. Immunoprecipitation of NDPK with a monospecific polyclonal immuneserum pulls down another distinct polypeptide. The most abundant NDPK interactor after IMAC has been identified as an *Arabidopsis* cytosolic NDPK isoform by automated Edman sequencing, and by immunoblot analysis indicating that the tagged protein forms oligomers with the resident enzyme *in vivo*. Preliminary data obtained by immunoblot analysis identify a 37 kDa NDPK interactor as a cytosolic glycolytic enzyme. This protein and other NDPK interactors are blocked at the N-terminus. Cold treatment (4 and 24 h at 4°C) and Pi starvation (4 and 7 days at 0 mM Pi) do not affect the the identity of these interations. We are currently assessing the occurence of protein interactors with control roots overexpressing (6xHis) cytosolic triosephosphate isomerase and (6xHis) cytosolic glyrealdehyde-3P dehydrogenase. Cold treatment (4 and 24 h at 4°C) and Pi starvation (4 and 7 days at 0 mM Pi) do not affect the identity of these interations.

7. To further identify and genetically map genes involved in nutrient use efficiency in *Brassica napus*. Status: Genome Prairie-supported research terminated. Funding provided by ACIDF. In 2005, we received funding from the Alberta Crop Industry Development Fund (ACIDF) to continue our work on NUE in canola. We are currently reassessing the germplasm we evaluated in 2003 and 2004 in field trials at the U. of A. Ellerslie Research Station. The mapping population chosen segregates for a large number of characters including days to flower, days to harvest, plant height, plant biomass, pod size, seed size, and of course seed yield. Because seed of this population was limited we were only able to conduct single row comparisons in 2004. However, seed from all lines are currently evaluated. We intend to continue to conduct more intensive phenotypic analysis on lines that show extensive differences, including leaf area measurements, growth rate analysis and N leaf content. This observations may be helpful in calibrating fertilizer recommendations. We have also included in our analysis this summer, the parents of an interspecific cross between *B. napus* and *B. carinata* that segregates for a number of traits.

8. Biochemical analysis of transgenic plant culture expressing varying levels of a class I hemoglobin to understand its role in managing hypoxic stress. **Status: Genome Prairie-supported research terminated. Objective a)** Several enzymes and metabolites associated with the ascorbate/glutathione cycle have been measured in Hb-transformed alfalfa lines. Lines over-expressing Hb have higher ascorbate levels, higher ascorbate peroxidase, lower glutathione reductase and lower dehydroascorbate

peroxidase than Hb-antisense lines under both aerobic and anaerobic conditions, indicating that plants expressing Hb have greater capacity to handle reactive oxygen species (Manuscript submitted) **b)** Evidence of Hb participation in an NO-dioxygenase-type reaction has been established (Planta 219:95 (2004)). Using site-directed mutagenesis, it has been established that an unique Cys in Hb is not involved in the NO-dioxygenase reaction, but that it is involved in protecting the Hb-Fe from auto-oxidation and in maintaining the tertiary and quaternary structure of the molecule (Manuscript submitted). A review on this topic has been submitted (J Exp. Bot **55:** 2473 -2483 (2004)). **c)** A collaborative project with groups in Poland and Hungary has shown that Hb over-expression in tobacco plants results in enhanced expression of PR-1 protein upon pathogen infection (FEBS Letters **571:** 61-66 (2004)). **d)** Transgenic alfalfa plants over-expressing Hb show strong phenotypic differences from control plants. They have pronounced apical dominance both in roots and shoots, elongated petioles, petiolules and leaves with increased leaf chlorophyll, differing flowering color and shorter flowering times, and increased susceptibility to drought stress due to interference with stomatal closure. The results are indicative of interference in hormone signal transduction pathways due to NO breakdown by Hb (J Exp Bot – submitted Jan 2005).

Remarks and Highlights

Reproducible 2-D maps for the extracytosolic proteins have been established. Tandem mass spectra generated using capillary LC-MS/MS and Q-TOF (Micromass) MS with a CapLC HPLC are used for de novo peptide sequence determination. .

There is a large body of evidence that implicates primary metabolism in root responses to environmental stresses and it is thought that protein-protein interactions play an important role in controlling these responses. 6xHis-tagged proteins/enzymes of primary metabolism are being over-expressed in roots to use as bait to affinity-purify interacting protein partners under various in planta conditions. The strategy begins with identification of full-length cDNAs followed by preparation of genetic constructs encoding 6xHis-tagged proteins. Plasmids containing these constructs are used to transform plants using Agrobacterium rhizogenes. Purified interacting proteins from control and stressed root tissue are purified by affinity chromatography and analyzed by MALDI-TOF MS.

This group has been working to develop stronger national and international relations, and also to refocus their efforts to only examining Pi stress. The group will attempt to publish the information on other nutrient stresses to date. Dr. Muench will complete his involvement with the nutritional stress studies and will redirect his studies to cold tolerance and signaling. Dr. Rivoal will examine protein:protein interactions of lead candidate gene products focusing on Pi stress with an interactome probing system. He will also work in collaboration with Quebec-based GP researchers to address protein:protein interactions in cold tolerance in cereals.

D. ELUCIDATING THE ROLE OF ABA IN ABIOTIC STRESS

Overall Goals

- To investigate the role of ABA in regulating pathways involved in cold and drought tolerance.
- To identify and characterize ABA binding proteins.

Contributing Groups

- Abrams (ABA analogue synthesis, affinity method development and application, NMR/MS analysis)
- **Cutler** (characterising ABA signalling using ABA analogues and microarrays, drought)
- Hill (ABA binding protein identification)

Objectives and Outcomes

1. Measure gene expression changes in Arabidopsis under drought stress and in response to ABA and select ABA analogues. Status (physiology and microarrays): 100% Complete. Physiological assays have been completed. Hybridization protocols have been optimised for oligo and cDNA arrays (these protocols have been transferred between labs) and analyses of gene expression at time intervals following treatment with (+)-ABA, (-)-ABA and the ABA analogues PBI425 and PBI51 have been completed. In addition, gene expression has been analyzed at intervals during soil drying to identify drought-associated genes. More than 500 genes differentially expressed in response to ABA treatment have been identified and these genes, and associated patterns of expression, are being compared to genes and expression patterns

observed during drought stress. Objective (March 2004): To complete hybridization experiments. Status: 100% complete. In response to mid-term review comments, we have given a higher priority to identifying gene targets and candidates have now been identified for detailed functional studies. These genes include several putative phosphoprotein phosphatases, a myb-type transcription factor and a gene coding for a putative cell wall enzyme that exhibits an unexpected pattern of expression. Objective (April 2005): functional analysis of at least one target gene. Insertional knockouts for these target genes have been obtained (Salk Institute) and homozygous lines prepared. Phenotypic studies are under way - we are examining these lines for visible (morphological) effects, altered ABA responses and stress tolerance. A protein phosphatase knockout line has been studied intensively and exhibits a pronounced insensitivity of stomatal aperture to exogenous ABA, however there was no change in germination rate with or without ABA treatment. . Where appropriate, we will apply hormone metabolite profiling and microarray analyses of changes in gene expression to further define mutational effects. In addition, we have studied the function of a putative cell-wall related gene and a putative transcription factor by analysing knockout phenotypes. Status: 80% complete. We are preparing manuscripts for publication.

2. Utilize custom array of 600 cDNAs from Brassica and Arabidopsis involved in response to ABA and drought stress, plant metabolism, lipid biosynthesis and signalling. Status: Complete. Custom array used in several preliminary experiments. Objective (March 2004): complete hybridization experiments as for objective 1. Status: 100% Completed.

3. To identify and characterize ABA-binding proteins in plants using biotin conjugated ABA analogs. Status: 70% Complete. Synthesis of first tethered bioactive ABA analogs achieved, synthesis and applications for affinity chromatography and covalent labeling ongoing. Using Mab raised against (+)-ABA as a model system for identifying ABA binding proteins by affinity chromatography, sepharose/avidin with bound analog biotin-tethered (+)-ABA shown to bind the Mab, and release on (+)-ABA addition. Objective (March 2004): Optimize affinity chromatography and synthesis of photo-affinity probes. 100% Complete. Affinity probes with tethered ABA analogs have been synthesized and demonstrated to bind proteins that recognize ABA. The (+)-tetralone probe was found to be active in an assay in MD embryos of B. napus, in which both (+)-ABA and the (+)-enantiomer of the ABA analog employed in the probe induce the message for a fatty acid elongase gene (with D. Taylor and A. Jadhav, PBI). A manuscript describing the synthesis and validation of the affinity probes has been published in the Journal of the American Chemical Society 2005.Manuscripts on the synthesis and biological activity of the tetralone ABA analogs and also on the corresponding hydroxymethyl derivatives are in preparation. A column sepharose-avidin with the (+)-tetralone probe was provided to the Hill Lab and found to bind both ABAP1 and FCA from Arabidopsis. Objective October 2005 80% complete): An ABA-binding protein from Arabidopsis has been isolated and purified using the affinity probe. The cytochrome P450 monooxygenase CYP707A1 gene was transformed, overexpressed in yeast and shown to have ABA 8-hydroxylase activity (HPLC analysis of ABA to PA conversion). The protein was retained by the affinity probe on the sepharose column and eluted with +ABA. Purification by SDS-PAGE and digestion of the protein with trypsin followed by analysis by MALDI ToF gave matching peptide sequences covering 69% of the protein, confirming identity. Experiments in progress include resynthesis of tetralone affinity probes and application to isolation of proteins from Arabidopsis. The structural requirements of the ABAP1 binding protein are being probed using ABA analogs (tetralones without tether) to identify molecules that can be best used for photoaffinity labeling studies. Testing is being performed by Hill lab. Studies are now underway to identify ABA-binding proteins from Arabidopsis, first looking for soluble proteins that bind to the tetralone probe attached to the affinity column. These experiments are in progress. Synthesis of one potential photoaffinity probe for identification of the active site of the ABAP1 isolated by the Hill lab has been completed. This probe is altered at the carboxyl terminus as the ABAP1 binds ABA and the aldehyde and alcohol. Attempts to isolate new ABA-bonding proteins are underway. Using affinity methods we found several proteins binding ABA-based probe from both cytosolic and membrane fractions of *Arabidopsis* wild type leaf tissue. Methods for identification of isolated protein are currently being developed and optimized. We are using two approaches to identify new ABA-binding proteins: Streptavidin affinity column modified with ABA-probe and second: photoaffinity labeling of unknown ABA-binding proteins using the same probe, followed by purification on streptavidin-biotin affinity column. In the first photoaffinity experiments using microsomal preparations from Arabidopsis leaf tissues, several proteins were labeled. The proteins are being isolated and subjected to mass spectrometric analysis using MALDI. Set of unknown proteins isolated using both techniques will be compared by their sequence and their ABA-binding ability will be further investigated.

4. To characterize an ABA binding protein from plants. **Status**: **Completed.** An ABA binding protein (ABAP1) has been shown to bind (+)-ABA and corresponding alcohol and aldehyde, but not catabolites of ABA nor (-)-ABA. It binds ABA very tightly (K_d = 28 nM) in a manner physiologically consistent with known ABA effects [J Biol Chem 279: 9922 (2004). Evidence has been obtained to suggest that reactive oxygen species inhibit binding of ABA to ABAP1, possibly through oxidation of an SH group. We are withholding submission of these results until the work can be placed in better context to the function of ABAP1. A manuscript has been accepted in Nature demonstrating that an RNA binding protein (FCA), involved in the transition to flowering, binds ABA. Binding of ABA to FCA interferes with the interaction of FCA with a nuclear transcription factor, FY. Because of this, there is no repression of FLC, which prevents the transition to flowering. This is the first demonstration of a physiological action involving a characterized, ABA hormone receptor. The work was in collaboration with G. Simpson, Dundee University and C. Dean, John Innes Institute, Norwich.

FCA binds biotin-tethered (+)-ABA weakly and does not bind ABAP1. Competition studies with an ABA analog, from which biotin-tethered ABA was prepared, show that it is not an effective competitor of ABA for the ABAP1 binding site. An acetylenic ABA analog is an effective competitor of ABA binding to ABAP1. Further testing of analogs will continue. We are also planning to initiate proteolytic digestion studies with ABAP1 and FCA using ABA to protect the active site.

In collaboration with the Australian Plant Genomics Centre, barley mutants lacking ABAP1 are being generated using RNAi protocols. André Laroche is also preparing mutants using his protocol. Fathey Sarhan has screened the wheat cold stress library and identified ESTs coding for ABAP1 within the library and has preliminary evidence through yeast two hybrid analyses that ABAP1 may interact with proteins involved in vernalization. Further work is planned to confirm this interaction. Sarhan will also work to identify the promoter region of ABAP1.

Determine the nature of the ABA binding site in an ABA binding protein. **Status: 80% Complete. a)** Constructs comprising a ~10 kDA and a 20 kDa fragment of ABAP1 have been prepared and the expressed proteins shown to bind ABA. We are attempting to prepare ABAP1 in sufficient quantities to crystallize the protein for x-ray crystallography by Drs. Peter Loewen and Dr. Brian Mark, Microbiology, University of Manitoba. We are encountering difficulties maintaining the protein in solution at high concentrations. Extend experiments to further localize the site of ABA binding in ABAP1.

Characterize the interaction between ABA45, ABAP1 and downstream components of ABA signal transduction. We have identified a barley aleurone protein, ABA45 that interacts with ABAP1. ABA45 has a GRAM domain, suggesting that it is located in membranes, a peptide motif that interacts with WW domains (ABAP1 has a WW domain) and SH2 and SH3 binding domains that interact with protein kinases. Dr Muench's lab has demonstrated that ABAP1 and ABA45 locate to the plasma and nuclear membranes of onion epidermal cells when the cells are transformed to express the proteins as fluorescently-tagged fusion proteins. ABAP1 transcript levels are high in embryos of McLeod barley and low in Harrington embryos. Germination of McLeod embryos is inhibited by ABA concentrations as low as 1 μM whereas Harrington embryos continue to germinate at concentrations as high as 100 μM. ABAP1 transcription is induced by ABA and inhibited by GA. The results suggest that ABAP1 may be acting as a hormone receptor relative to germination and dormancy.

Remarks and Highlights:

The FGAS project can now lay claim to funding the first description of an ABA hormone receptor protein. In a Nature paper, we describe that FCA, an RNA binding protein involved in flowering, binds ABA with high affinity, in an interaction that is stereospecific and follows receptor kinetics. The FCA-ABA binding has molecular effects on downstream events in the autonomous floral pathway and, consequently, on the ability of the plant to undergo transition to flowering. ABA binding exerts a direct control on FCA pre-mRNA processing. The results indicate that FCA is an ABA receptor involved in RNA metabolism and flowering time control. This work lends strong support to the likelihood that ABAP1, a barley protein whose function is much more difficult to establish and which shares many common features with FCA, is also an ABA hormone receptor protein. There is also a strong possibility that the two initial proteins in cereal grain ABA signal transduction may be ABAP1 and ABA45, as ABA45 interacts with ABAP1

A physiological assay to measure growth of *Arabidopsis* plants under prolonged drought stress has been developed and optimized. The effects of applications of (+)-ABA have been compared to a range of ABA

analogs at various concentrations; several of the applications produce a significant improvement in plant growth under drought stress. A series of array hybridizations using labeled probes from both well-watered plants and plants in drying soil, and treated with ABA and select analogs (3 to 48 h) and untreated plants in drying soil have been completed. Detailed data analysis has allowed us to identify gene targets for detailed functional studies (see above). This data will be compared with similar data being derived in Theme I, describing the acclimation response in *Arabidopsis*.

III. PLATFORM TECHNOLOGIES

Platform technologies refer to a suite of dedicated technologies that have been implemented to provide strategic-level technical support to the FGAS themes and sub-projects. The platform technologies implemented by the FGAS project reside largely in Saskatoon and Edmonton, where project personnel with the required experience in high-throughput methods and informatics are based.

For both the wheat and *Brassica* theme groups, platform technologies have been strongly directed by the sub-projects to which they provide an essential service, such that a platform group focused to *Brassica* (Parkin) and Wheat (Crosby) are being operated. In both themes, DNA sequencing, proteomics, Bioinformatics and array development form the core suite of technologies making up the platform. Early in its implementation, the platforms have worked to implement the necessary tools required for downstream functional genomics experiments, and these tools are mostly specific to the experimental systems under study with the exception of Bioinformatics and microarray manufacture. It follows that Bioinformatics and array development activities constitute the principal interface between the *Brassica* and Wheat components, with other aspects being operated at the level of fundamental protocols and methods.

A. DNA (EST SEQUENCING)

Objectives

- Organize and implement a high-throughput, responsive and cost-effective sequencing capability on behalf of the EST sequencing needs of the project. Status: **Complete**
- Apply the technology developed to the prosecution of FGAS EST sequencing goals in wheat, namely, the production of 80,000 'quality' (PHRED > 20) sequence runs for the wheat EST project. Status: **Complete**; as stated elsewhere, approximately 80,000 quality runs were deposited to the public data repertoire on Dec 3rd, 2003.

Contributing Groups

- **Crosby** (Sequence reactions, quality control)
- Pelcher (capillary sequence reaction resolution)
- **Ross** (proteomics)
- Haughn (TILLING, mutant analysis)
- Parkin (Bioinformatics, SAGE analysis, sequencing, TILLING)
- P. Lepage (Montreal Genome Centre, Genome Québec, Montreal; capillary sequence reaction resolution)
- B. Olfason (U. Victoria Proteomics Facility; ICAT-MUDPIT Proteomics)

Remarks and Highlights

In January of 2003 the group reviewed its sequencing needs against available technology and throughput, resulting in the decision to consolidate activity to the USask. By April, sequence reactions for the backlog of library samples were completed.

Since implementing new lower-cost protocols at the USask, the frequency of 'quality' runs produced by the group has grown from ~65% (November) to the current ~88%.

The wheat sequencing platform operates no sequencers of its own; finished sequence reactions are resolved in working collaboration with the Montreal Genome Centre (MGC) and the NRC-PBI on a cost-recovery basis, with each Institution providing about 50% of the projects sequencer outlet needs thus far.

Protocols and group organization have matured to the point that, with 1 day preparation, two personnel can prosecute 10,000 finished sequencing reactions without automation. This capability can fully support the projects sequencing requirements on a part-time basis. As of November 2003 approximately 106,000 total reactions have been conducted, and will result in over 80,000 quality sequence data runs by December 2003 – 3 months ahead of project schedule.

The proposed EST sequencing program for wheat has been completed to the extent and to a quality standard defined by the Mid-Term Review process in June of 2003. This aspect of the program was completed 3 months ahead of schedule.

B. PROTEOMICS

Objectives

- Acquire, install and optimize infrastructure for high-throughput protein identification at PBI. Status: **Completed**.
- Hire technician for excision, tracking, processing and MS analysis of proteins. Status: **Completed**.
- Optimize MALDI MS procedures for protein identification. Status: **Completed**.
- Optimize Q/TOF MS/MS procedures for protein identification. Status: **Completed**.
- Optimize procedures for identifying and mapping posttranslational modifications. Status: **Ongoing**.
- Provide processing, MS analysis and data interpretation for protein samples. Status: **Established and Ongoing**.

Contributing Groups

- **Ross** (acquisition of proteomics infrastructure; development and implementation of MS-based methods for protein identification)
- **Gray** (provision of protein samples)
- **Gusta** and Weselake (*ibid*)

Remarks and Highlights

The goal of this project was to set up a Proteomics Platform at the NRC Plant Biotechnology Institute to support FGAS research. Initial work focused on optimizing procedures for protein identification. Standard protein mixtures were prepared, resolved by 2-D gel electrophoresis, automatically excised and processed for MS analysis.

The first GP samples were subsequently received from Dr. Gray's group (USask), while Dr. Gusta's group (USask) was given access to equipment for acquiring and analyzing gel images in order to target differentially expressed proteins.

All our project milestones have been met, and a paper describing the evaluation of our automated protein excision and digestion procedures has been published (Ross et al. 2002, Proteomics 2, 928-936). Techniques for characterizing phosphorylated proteins have also been developed.

As a result of the decision by the Wheat group to divert resources from gel-based proteomics to ICAT-MuDPIT analysis, the technician originally hired to provide protein MS analysis and method development for FGAS researchers has now been terminated (effective August 1, 2003), along with all operational support for the Proteomics Platform. PBI's mass spectrometry facility continues to provide LC-MS/MS identification of gel-separated proteins and profiling of plant hormones for *Brassica* researchers on a fee-for-service basis, and is currently engaged in ICAT-MudPIT method development in collaboration with Dr. Gusta's group, with PENCE and NRC funding.

C. TILLING

Objectives

- Establish a high quality TILLING operation in *Arabidopsis* and demonstrate robustness of the technology as a Genome Prairie platform component. Status: **Complete**
- Perform TILLING of an *Arabidopsis* population on request of researchers in the Functional Genomics of Abiotic Stress Program. Status: **Complete**
- Use TILLING to screen mutagenized *Brassica oleracea* DNA library. Status: **Ongoing**

Contributing Groups

- Haughn (TILLING, mutant analysis)
- Parkin (Bioinformatics, *Brassica* TILLING)

Remarks and Highlights

We have requests from GP researchers to TILL eleven genes in *Arabidopsis* including some of the genes Isobel Parkin is using for RNAi tests. To date, mutations in eight genes have been sequenced, and seed stock information for 103 different mutations has been provided to the requestors. Primers have been designed for all remaining requests and mutations that have been identified are currently being sequenced.

Table 4: *Arabidopsis* Requests for TILLING

AGIname	PI	Description	Num. Mutations
At1g17290	Good, Allen	alanine aminotransferase	14
At1g29390	Parkin, Isobel	unknown protein	17
At1g29395	Parkin, Isobel	unknown protein	16
At1g72330	Good, Allen	alanine aminotransferase	10
At1g73600	Parkin, Isobel	phosphoethanolamine N-methyltransferase, putative	9
At1g80640	Good, Allen	serine-threonine protein kinase	6
At2g15970	Parkin, Isobel	similar to cold acclimation protein WCOR413 [Triticum aestivum]	6
At2g23680	Parkin, Isobel	similar to cold acclimation protein WCOR413 [Triticum aestivum]	8
At3g18000	Parkin, Isobel	methyltransferase, putative	12
At3g50830	Parkin, Isobel	COR413-PM2 cold acclimation protein WCOR413-like protein beta form.	19
At5g58070	Parkin, Isobel	outer membrane lipoprotein - like	12

We have completed screening of requests for Arabidopsis mutations and sent seed stock information to requestors.

The Parkin lab (AAFC, Saskatoon) has sent DNA extracted from 960 *Brassica oleracea* EMS lines to the TILLING lab (Haughn, UBC) and preliminary screening indicates that TILLING works well in this species. We have obtained genomic sequence information for 31 *B. oleracea* homologues of genes reported to be involved in abiotic stress response in *Arabidopsis* and have designed primers to these. We have begun TILLING on eight of these genes and identified mutations in of these. We have sequenced all the mutations identified in a glucose-6-phosphate isomerase *Brassica oleracea* homologue and identified two missense mutations that are likely to affect the function of the protein.We are awaiting requests from Genome Prairie researchers for additional genes to be TILLed in *B. oleracea*. We ask that requestors submit genomic DNA sequences of greater than 1000 bp (preferably >1500 bp) to us in order to design primers that are appropriate for TILLING. They may instead submit the name or sequence of the homologous gene in *Arabidopsis* and we will design primers to the *B. oleracea* gene based on this if genomic sequence is available. A list of *B. oleracea* genes currently being tested or screened is below (Table 5):

Amplicon	Arabidopsis homologue	Description	Status
bo2	At5g42740	PgiC gene - glucose-6-phosphate isomerase	4 mutations (2 deleterious)
bo3	At1g17920	alanine aminotransferase	primers give multiple products
bo4	At1g29390	COR314-TM2 encodes a protein similar to the cold acclimation protein WCOR413 in wheat.	primers give multiple products
bo5	At3g18000	methyltransferase, putative	testing new primers
bo6	At5g58070	outer membrane lipoprotein - like	testing new primers
bo7	At1g72330	alanine aminotransferase	not enough genomic sequence to

Amplicon	Arabidopsis homologue	Description	Status
			design good primers
bo8	At2g15970	COR413-PM1- alpha form similar to cold acclimation protein WCOR413 in wheat.	testing new primers
bo9	AT1G08930	ERD6 early-responsive to dehydration stress protein (ERD6) / sugar transporter family protein	testing new primers
bo10	AT1G32230	CEO1 Can protect yeast from oxidative damage.	testing new primers
bo11	AT1G34000	light stress-responsive one-helix protein (OHP2)	testing new primers
bo12	AT1G62320	early-responsive to dehydration protein-related / ERD protein-related, similar to ERD4 protein	testing new primers
bo13	AT1G62740	stress-inducible protein, putative, similar to sti (stress inducible protein)	testing primers
bo14	AT1G71860	PTP1 - protein is downregulated in response to cold and upregulated in response to salt stress.	testing new primers
bo15	AT1G74310	ATHSP101 heat shock protein involved in thermotolerance to high temperature stress	testing primers
bo17	At1g35720	ANNAT1- member of the annexin gene family of calcium-dependent, membrane-binding proteins.	testing new primers
bo18	At1g55920	SAT1 cytosol, serine O-acetyltransferase activity, cellular response to sulfate starvation, root, leaf	primers amplify mostly non-coding DNA
bo19	AT5G64030	dehydration-responsive protein-related, similar to early-responsive to dehydration stress ERD3	2 putative mutations
bo20	AT5G63980	SAL1 - encodes a bifunctional protein that is involved in the response to cold, drought, and ABA.	many background bands on TILLING gels
bo21	AT1G01470	LEA14 Encodes late-embryogenesis abundant protein whose mRNA levels are induced in response to wounding and light stress	0 mutations
bo22	AT2G21970	SEP2 stress enhanced protein 2 (SEP2) chlorophyll a/b-binding protein	testing primers
bo23	AT2G39750	dehydration-responsive family protein, similar to early-responsive to dehydration stress ERD3	not enough genomic sequence to design good primers
bo24	AT2G45750	dehydration-responsive family protein [Arabidopsis thaliana]	testing primers
bo25	AT3G16050	stress-responsive protein, putative, similar to ethylene-inducible protein HEVER	1 putative mutation
bo26	AT3G51250	senescence/dehydration-associated protein-related, similar to senescence-associated protein 12	testing primers
bo27	AT4G00750	dehydration-responsive family protein, similar to early-responsive to dehydration stress ERD3	testing primers
bo28	AT4G02380	SAG21 - late embryogenesis abundant 3 family protein - induced by heat, auxin, ethylene and wounding	testing primer
bo29	AT4G02900	early-responsive to dehydration protein-related / ERD protein-related, similar to ERD4	testing primers
bo30	AT5G06050	dehydration-responsive protein-related, similar to early-responsive to dehydration stress ERD3	testing primers
bo31	AT5G63310	NDPK2 - Maintains intracellular dNTP levels except ATP, also plays a role in oxidative stress	testing primers

Table 5: List of *B. Oleracea* genes tested for TILLING

D. BIOINFORMATICS

Objectives

- Implement a flexible back-end database system to store project-related data (including EST sequences, annotation and LIMS data) based on *MySQL*, a proven and popular open source relational database management system. Create a flexible interface to the underlying database using BioPerl, allowing primary data to be imported in a variety of standard formats. Status: **Completed**, and actively maintained
- Develop an automated EST analysis pipeline to screen incoming sequence data for quality and ascribe putative annotation, including a system to quantify the level of redundancy in the EST data set through clustering. Status: **Completed**, and actively updated
- Develop a secure web-based portal for remote access to project-related data, based on Perl, CGI and Java, providing access to data at various levels of processing from raw sequence to clustering and annotation data in one unified interface. Status: **Ongoing**; largely driven by user feedback
- Implement tools for the comparative evaluation of project data against that of model systems (*Arabidopsis*, Rice) with a view to maximizing functional information available to the crop data sets. Status: **Completed**; initiated in March in response to a Genome Prairie recommendation, completed in January of 2004 in continuing collaboration with the Bioinformatics component of the *Brassica* theme group (Parkin, AAFC-SRC).

Contributing Groups

- **Crosby** (Bioinformatics, Wheat-Rice, Wheat-*Arabidopsis*)
- Parkin (Bioinformatics, *Brassica-Arabidopsis*, Wheat-*Arabidopsis*)

Remarks and Highlights

The Informatics implementation provides users with various means to query/browse the data through the web interface, including searches based on sequence homology, annotation, etc. The security of project-related data is ensured by wrapping the web interface in a modern authentication and access control system based on published best practices for secure programming.

The group has completed design of an initial 10,000-feature microarray representative of the EST repertoire through the identification of signature oligonucleotides. This aspect will be expanded to provide for an enlarged array (10,000 additional features), as required by the project

Deployment of the TM4 microarray analysis package and integration with the existing web interface (TIGR) to provide access to the EST and LIMS data is underway, and targeted to achieve functional status by Fall 2003 - when array data is anticipated to begin accruing to the project. Initial implementation of the system has been completed (Nov, 2003)

The group has initiated construction of a system for the warehousing and analysis of proteomics data, facilitating the comparison of data from different experiments, and against heterologous model systems. This has involved close cooperation with the 'user' groups, particularly Gray who is providing processed data to map mass-charge fragments to a custom plant-only dataset.

In combination, the group presents six highly experience individuals who command over 16 person-years of experience in Bioinformatics-related application development, database implementation, systems administration and web-based portal development. This experience is strongly centred on the use of Lin/Tel and Open Source solutions, which form the backbone of the rapidly developing, public domain Bioinformatics community.

The project systems that allow remote access and querying of central datasets are largely mature, although the interface features continue to undergo evolution in response to user requests.

Through an expanded international collaboration involving the Australian CRC network and two American laboratories, the group has completed design of a 17,300-feature microarray representative of the EST repertoire through the identification of signature oligonucleotides. Delays were incurred through Jan-Feb in order to consolidate data input from all partners, leading to placement of an oligo manufacturing order in mid-March, with a view to manufacturing test arrays by early-mid April. The international array consortium has been recently expanded to include Dr. Keith Edwards (U. Bristol, UK)

upon his success in securing BBSRC funding. We anticipate this will boost the complexity of the international array from ~17,000 features to a total of 25-30,000 probes. Deployment of the TM4 microarray analysis package is completed for the Wheat theme group, and is ready for use as test-array data begins to flow – anticipated for late April 2004. Finally, the group has deployed a simple system for the warehousing and analysis of proteomics data, facilitating the comparison of data from different experiments, and against heterologous model systems.

September of 2004 saw completion of the relocation of the Crosby lab from Saskatoon to the University of Windsor. The sole remaining member of the project group under FGAS support (Mr. Matt Links) likewise relocated to Windsor in September along with the Bioinformatics wheat-related server and support component of the project. Mr. Links will be supported through the remaining available Technology Platform funds, which will be exhausted at the end of January 2005.
The project servers were packed, shipped and redeployed to temporary facilities at the University of Windsor, spanning a timer interval when a working server was maintained at the University of Saskatchewan in order to provide for non interruption in access to the data. Project data repositories and Web site have been re-deployed at the new URL http://bioinformatics.uwindsor.ca.
All Windsor-based servers were up and operational in October, along with migration of the InterPro data from the USask computing infrastructure (Cluster facilities; HMMER application use for defining a final assembled EST set).

By way of computational analyses, the remaining InterPro runs were completed up to the final integration step, which will be completed on UWindsor cluster facilities in the coming quarter. In support of the project, and a request from the Montreal-based group, a complete copy of all 'quality' EST sequences were forwarded to the University of Calgary Bioinformatics Centre (Dr. Paul Gordon) for additional analysis against the globally available EST repertoire from wheat using HPC facilities resident there.

The group continues to work toward distributing an array of acceptable quality. In concert with a decision by the Wheat sub-group of PIs, oligonucleotide and informatics materiel were transferred to Dr. Gulick and to Microarray Inc. (USA) for the production of wheat arrays from the latter printing facility. After extensive discussion, it was felt that further exploration of the UAlberta and/or the UToronto facilities was a less favourable option. Advantages relating to coordination with US-based partners (USDA-ARS, Berkeley) was cited as a major reason to print arrays via the US-based facility. These arrays will be assessed by the AAFC-LRC and Montreal-based user groups for their experimental acceptability.

Arrangements were completed for a transfer of micro-array related data to members of the group and to off-site printing facilities as outlined above. A general plan within which server accessibility will be maintained at the UWindsor into the near- to medium-term was agreed. Discussions are ongoing for the long-term archiving of project informatics-related data – possibly via the CBR, or other Bioinformatics centre or platform (e.g. the UCalgary facility).

The Platform participants have no remaining issues to address. The opportunity to serve the FGAS project has been an instructional and rewarding experience. We will endeavor to stay in contact, particularly on Bioinformatics-related matters, on a low-key basis and as the need arises.

All the wheat data from the Windsor server was copied to hard drives and transferred to Saskatoon Research Centre. The drives are presently being set-up in Saskatoon and will be maintained as required.

Work to deploy a simple system for organizing and querying *Brassica* related data through a secure Web site was developed at the AAFC (Saskatoon). The site allows data to be deposited and displayed for each of the participating researchers that will allow comparisons to be made between the different data sets. Microarray data (.MEV files) from the analysis of cold acclimation in *Thlaspi arvense* and from the analysis of phosphate starvation in *B. napus* has been deposited on the website. Microarray data exploring the response of plants to drought stress have now been deposited on the website. The accumulated data is in the process of being compared.

A paper describing the *Brassica*/*Arabidopsis* genome viewer developed at AAFC has been published (Christopher T. Lewis, Andrew G. Sharpe, Derek J. Lydiate and Isobel A. P. Parkin. 2003. The *Brassica*/*Arabidopsis* Comparative Genome Browser: A novel approach to genome browsing. J. Plant Biotech. 5(4): 197-200). The *Brassica*/*Arabidopsis* genome viewer has been adapted to display the wheat

EST clones which will allow the project members to identify interesting genes which potential span the monocot-dicot divide. the present viewer which is publicly available will be updated with wheat ESTs in mid-October.

The SAGE data generated from the *Brassica* group presented some interesting bioinformatics problems, with the identification of a number of novel transcripts. The analysis of this data has been accepted for publication in the Bioinformatics section of Plant Physiology (October issue). There is also now a public website with access to the SAGE data (http://www.*Brassica*.ca/SAGE).

A bioinformatics student was hired over the summer and has been developing tools to convert the available expression data into a common format based on Arabidopsis gene codes. This will assist in comparing data from studies in all species.

IV. PROJECT INTERACTION AND INTEGRATION OF RESEARCH THEMES

As the Science section outline would attest, most FGAS sub-projects are achieving their goals through the cooperative interaction of two or more laboratory groups. This operational reality underpins the project, and is entirely consistent with the rationale for large genomics projects of the sort supported by Genome Canada - the capture of synergy and associated scientific benefits through the focused cooperation of leading researchers in the field.

In more specific terms, the FGAS project has been heavily committed to completing its program of 'tool implementation' on behalf of the project. This will have two positive effects on group interaction: i) the provision of common resources as the substrate for experimentation (e.g. comprehensive *Arabidopsis* arrays), permitting direct comparisons between different species and systems within the project, and ii) migrate both theme area activities in the direction of elucidating biological mechanisms, where scientific common ground is likely to be identified and shared, leading to new inter-project and inter-theme interactions.

A strong precedent for the development of common inter-theme objectives has been in the area of Bioinformatics. Both theme groups have met under the auspices of the Steering Committee to exchange new ideas and directions. This activity has identified a common software and implementation strategy between the *Brassica* and Wheat platform groups for the management of microarray-based experimental data. The group has completed implementation of the TIGR TM4 suite as a common platform for managing microarray data, as a cooperative effort of a joint project team. This work included the scripting and forwarding of patches to existing versions of the suite (e.g. for remote Dbase access), as a contribution to further development of this Open Source resource. The joint working group has also identified opportunities and discussed working priorities for the cross-referencing of function-related data from *Arabidopsis*, Rice and the two crop systems under study by the FGAS project. This cross-referencing effort includes the analysis of Proteomics data – particularly that arising within the wheat theme. Implementation of a cross-model (functional) data comparative system was started in September 2003, with the view to implementing at least a demonstration capability during the fall of 2003. The two data sets (sequence contigs with proteomics) have now been integrated and can be co-viewed via the Project informatics web site. A complete InterPro analysis (for motif content, and predicted function) for the project UniGene set is nearing completion as a large computational task (~80 days using existing parallel computing resources), and is scheduled for completion in July. When completed in August, this data will be incorporated and made available to project users via the integrated Web portal.

E. EQUIPMENT

There was no budget for equipment.

F. PROJECT DEVELOPED MATERIALS

To date, the project has developed several strategic tool sets that have found application within the project, and have served as the substrate for international collaboration with suitably complementary programs in Australia and the United States. These are:

1. 80,000 quality (PHRED score $\geq$ 20) wheat EST sequences repertoire that incorporates approximately 27,000 genes.
2. Development of 628 doubled-haploid (DH) lines from Norstar X winter Manitou and Norstar X Manitou (spring).
3. Construction of a BAC library containing 1.27 million clones from the hexaploid cv 'Norstar' – a specialized wheat germplasm selected for cold tolerance.
4. Effective completion of a specialized 82,150-run 'quality' EST data set, in support of the wheat cold-acclimation research theme, and mining of this data set for lead-candidate genes of possible importance to the regulation of cold tolerance in temperate cereals.
5. Bin mapping of 600 FGAS EST clones.
6. Mapping of almost 900 SSR and AFLP markers mapped on different wheat populations.
7. Development of 17,300- features wheat microarrays.
8. Microarray resources representing the full genomic compliment of genes from *Arabidopsis thaliana*, encompassing approximately 25,000 genes.

9. Generation of 400,000 Arabidopsis leaf tissue SAGE tags which were deposited in GenBank under the accession number GSM30396 (http://www.ncbi.nlm.nih.gov/geo/).
10. 122 microtubule binding protein sequences were deposited into BIND database.

Referred Publications

- Trischuk, R.G., B.S. Schilling, M. Wisniewski, and **L.V. Gusta** (2006) Freezing Stress. In: Physiology and Molecular Biology of Stress Tolerance in Plants. Madhava Rao, K.V.,Raghavendra, A.S., Janardhan Reddy, K. (Eds.) Kluwer Academic Publishers.
- Kane, N.A., Danyluk, J., Tardif, G., Ouellet, F., **Laliberté, J.F.,** Limin, A.E., **Fowler, D.B.** and **Sarhan, F.** (2005) *TaVRT-2*, a member of the *St*MADS-11 clade of flowering repressors, is regulated by vernalization and photoperiod in wheat. Plant Physiology 138:2354-2363.
- Nyangulu , J.M., Galka, M.M., Jadhav, A., Gai, Y., Graham, C.M., Nelson, K.M., **Cutler, A.J.,** Taylor, D.C., Banowetz, G.M. and Abrams, S.R. (2005) Affinity Probes for Isolation of Abscisic Acid-binding proteins. JACS 127 1662-1664.
- Ratnayaka, I., Båga, M, **Fowler, D.B. and Chibbar, R.N. (2004)** Construction and characterization of BAC library of a cold-tolerant hexaploid wheat cultivar Crop science Crop science 45: 1571 – 1577.
- **Chibbar, R.N.,** Ganeshan, S., Båga, M. and Khandelwal, R.L. (2004) Carbohydrate metabolism. In Encyclopedia of Grain Science (eds. Wrigley, C., Corke, H. and Walker, C.E.) Elsevier U.K., pp. 168-179.
- **Fowler D.B.** and Limin A.E. (2004) Interactions among factors regulating phenological development and acclimation rate determine low-temperature tolerance in wheat. Annals of Botany 94, pp. 717–724.
- Appels, R., Francki, M. and **Chibbar, R.** (2003) Advances in cereal functional genomics. Functional and Integrative Genomics 3, pp.1-24.
- **Chibbar, R.N.** and Båga, M. (2003) Genetic Modification of Primary Metabolism – Carbohydrates. In Encyclopedia of Applied Plant Sciences (eds B. Thomas, D.J. Murphy and B.G. Murray) Elsevier U.K , pp. 449-459.
- Danyluk, J., Kane, N.A., Breton, G., Limin, A.E., **Fowler**, D.B. and Sarhan, F. (2003) TaVRT-1, a Putative Transcription Factor Associated with Vegetative to Reproductive Transition in Cereals. Plant Physiology 132, pp. 1849–1860.

Invited Conference Presentations

- **Crosby, W., Chibbar, R., Fowler, B., Gray, G.,** Gulick, P., Houde, M., Laliberté, J-F, Laroche, A., Pelcher, L., Sarhan, F., **Scoles, G.** and Varin, L.,
  Functional Genomics Of Abiotic Stress In Canada – Development Of Genomics Tools Toward Understanding The Impact Of Low Temperature On Wheat. Plant & Animal Genomes XIV Conference, San Diego, CA, January 14-18, 2006.
  Parkin, I., Abrams, S., **Crosby, W., Cutler, A.,** Good, **A., Gusta, L.** Haughn, G., Hill, R., Muench, D., Moloney, M., Rivoal, J., Taylor, G. and Weselake, R. Functional Genomics Of Abiotic Stress In The Brassicaeae Family. Plant & Animal Genomes XIV Conference, San Diego, CA, January 14-18, 2006.
- **Gray, G.R.**, Smith, D., **Fowler, D.B.** Limin, A.E., Kriger, J.N., Cherry, K.M., Shabaga, C. Proteomic analysis of the chloroplast reveals a specific role for photosynthesis in spring and winter cultivars of wheat. Proceedings of the 3rd Canadian Plant Genomics Workshop, Saskatoon, Saskatchewan, August 28-31, 2005.
- El-Khatib, R., Parkin, I., Abrams, S., **Cutler, A.**, Muench, D.G. Analysis of the Arabidopsis cell suspension phosphoproteome in response to short-term low temperature and abscisic acid treatment. Plant Canada 2005, Edmonton, AB, June 15-18, 2005.
- **Gusta, L.V.** The Identification of Cold acclimation Associated Proteins from Canola Utilizing conventional 2D-PAGE and 2 D-DIGE technologies. Functional Genomics of Abiotic Stress Meeting, Edmonton AB, Canada, June 14, 2005.

- Huang, D., Abrams, S. and **Cutler, A.** Investigating ABA and Stress-Related Gene Expressing Using ABA Analogs. Functional Genomics of Abiotic Stress Meeting, Edmonton AB, Canada, June 14, 2005.
- **Gusta, L.V.** and McKersie, B. (BASF Pl. Sci.) Effects of the CBF transcription factor on stress tolerance, gene expression and field performance of *Brassica napus*. Gordon Research Conference. Ventura, CA, Jan.29 – Feb.4, 2005.
- Shabaga, C., Propp, M.K., Baerr, J.N., **Fowler, D.B.**, Limin, A.E. and **Gray, G.R.** (2004) Photoinhibitory Responses in Wheat (*Triticum aestivum* L.) and Barley (*Hordeum vulgare L.*). CSPP Western Regional Meeting, Saskatoon Saskatchewan, December 9-10.
- **Gusta, L.V.** 2004. A "nomics" Approach to Studying Abiotic Stress and Seed Vigour in *Brassic* Presented at Canola Industry Meeting Saskatoon, SK Dec. 9 – 10.
- Båga, M. and **Chibbar, R.N.**) Biochemical and molecular characterization of genetic determinants of starch granule size in cereals. Starch granules – Size does matter. A symposium, American Association of Cereal Science, annual meeting, San Diego, California, USA, September 19-23, 2004.
- **D.B. Fowler**, Functional genomics of cold tolerance and crop adaptation. Canadian/German collaboration on plant research. Kananaskis AB, June 23-24, 2004.
- **Gusta, L.V.** Genomic and proteomic changes in *Brassica napus* in response to stress. Saskatchewan Agriculture and Food Rural Revitalization (SAFRR) meeting. University of Saskatchewan, Saskatoon, SK. December 112003.
- **Gusta, L.V.** Identification and characterization of novel proteins associated with frost tolerance development and over-wintering of *Brassica napus*. Genome Prairie Brassica Workshop. University of Saskatchewan, Saskatoon, SK. August 27, 2003.
- **Chibbar, R.N.** Biotechnology for crop improvement: Opportunities and challenges. Canadian Wheat Board conference: Herbicide Tolerant Crops: Weeding out the issues. The Saskatoon Inn, Saskatoon, SK. Canada, March 18 & 19, 2003.

Presentations at conferences

- Huang, D., Abrams, S., **Cutler, A.J.** Investigating ABA and stress related gene expression using ABA analogs. Presented at 18th International Conference on Plant Growth substances, Canberra, Australia, September 20-24, 2004.
- Chodaparambil, S.V., Båga, M., Limin, A.E., **Fowler, D.B.**, and **Chibbar, R.N.** Chromosomal regions of winter wheat participating in cold tolerance. Canadian Society of Agronomy annual meeting, Edmonton, Alberta, Canada July 20-23, 2004.
- Ganeshan, S., Leis, M., Jain, J. and **Chibbar, R.N.** An immature wheat spike culture system to evaluate the effects of abiotic stresses on grain development. Canadian Society of Agronomy annual meeting, Edmonton, Alberta, Canada July 20-23, 2004.
- S. Mahfoozi, **D. B. Fowler** and H. Ketata. Breeding and agronomic practices for winter survival in cereals. 1st International Caucasian conference on Winter Cereals Production, Tiblisi, Republic of Georgia. June 14, 2004.

Poster Presentations

- Ubayasena, L., Laroche, A., Gulick, P., Sarhan, F. and **Scoles, G.** Chromosome Bin Mapping Of Low Temperature Responsive Ests Of Wheat (*Triticum Aestivum* L.). Proceedings of the 3rd Canadian Plant Genomics Workshop, Saskatoon, Saskatchewan, August 28-31, 2005.
- Houde, M., Belcaid, M., Bergeron, A., Danyluk, J., Monroy, M., Gulick, P., Ouellet, F., Laroche, **A.**, Links, M., **Crosby, W.** and Sarhan, F. A wheat EST resource for cereal abiotic stress functional genomics. Plant Canada, Edmonton AB, Canada, June 15-18, 2005.
- Liu, X., and **Gusta, L.V.** Proteomic Analysis of Canola Seed Imbibed at 23°C and 2°C Functional Genomics of Abiotic Stress Meeting, Edmonton AB, Canada, June 14, 2005.
- Trischuk, R., J. Lorch, and **Gusta L.V.** Identification of Cold Acclimation Associated Proteins Utilizing 2D-DIGE. Functional Genomics of Abiotic Stress Meeting, Edmonton AB, Canada, June 14, 2005.

- Båga, M., Limin, A.E., Sanjay, V.C., Pecar, M., **Fowler, D.B. and Chibbar, R.N.** Genomic Mapping of Cold-Hardiness Genes in Winter Wheat. Functional Genomics of Abiotic Stress Meeting, Edmonton AB, Canada, June 14, 2005.
- Ratnayaka, I., Båga, M., **Fowler, D.B. and Chibbar, R.N.** Construction and Characterization of A Large-Insert BAC Library of Winter Wheat. Functional Genomics of Abiotic Stress Meeting, Edmonton AB, Canada, June 14, 2005.
- **Gray, G.R.**, Smith, D., **Fowler, D.B.**, Limin, A.E., McJannet, L., Thomas, J., Adams, S., Baerr, H., Baerr, J., Cherry, K., Hope, B., Jack, K., Olfert, J., Parent, J., Propp, M., Sauer, C., Shabaga, C., Su, J., Taylor, B. and Whitehead C. Proteomic Analysis of the Chloroplast During Cold Acclimation. Functional Genomics of Abiotic Stress Scientific Meeting, Edmonton, Alberta, June 14, 2005.
- Chodaparambil, S.V., Båga, M., Ratnayaka, I., Limin, A.E., **Fowler, D.B., and Chibbar, R.N**. Identification and characterization of a low-temperature tolerance QTL in winter wheat. 12th Annual Life Science Research Conference, University of Saskatchewan, Saskatoon, March 18, 2005.
- Ratnayaka, I., Båga, M., **Fowler, D.B. and Chibbar, R.N.** Characterization of wheat BAC clones carrying CBF genes associated with low-temperature tolerance QTL in wheat. 12th Annual Life Science Research Conference, University of Saskatchewan, Saskatoon, March 18, 2005. (Winner of the second prize (best poster) in the section 'Molecular Biology/Genetics/Genomics').
- Trischuk, R., Ruddat, V., Lorch, J. and **Gusta, L.V.** Proteome Differences in Canola Plants Subjected to Cold Stress, Cold Shock and Cold Acclimation. Gordon Research Conference Ventura, CA, Jan.29 – Feb. 4, 2005.
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- Schilling, B.S., Nesbitt, N.T. and **Gusta,** L.V. Physiological and Molecular Responses of Winter and Spring Canola During Cold Acclimation, De-acclimation, and Re-acclimation. Seventh Congress of the International Society for Plant Molecular Biology, June 2003, Barcelona, Spain.
- Liu, X., Gao, Y.-P., , Trischuk, R.G., Nesbitt, N.T. and **Gusta, L.V**. Freezing Tolerance of Imbibed Canola Seeds: 2D-PAGE of Seed Protective Proteins. Seventh Congress of the International Society for Plant Molecular Biology, June 2003, Barcelona, Spain.
- Trischuk, R.G., Liu, X. and **Gusta,** L.V. Unlocking the Cold Acclimation Process in *Brassica Napus*: 2D-PAGE of Heat Stable Proteins. Seventh Congress of the International Society for Plant Molecular Biology, June 2003, Barcelona, Spain.
- Ubayasena, L., Tomita, A., Eckstein, P., **Scoles**, G., Functional Genomics of Abiotic Stress – EST Deletion Mapping in Wheat. FGAS Project Site Visit, 2003, Saskatoon, SK.

Press Releases and Interviews

- **D.B. Fowler.** June 3, 2005 - Frost tolerant wheat - GX94 radio. Yorkton June 7, 2005 - Frost tolerant wheat - Hursh Consulting and Communications for Swift Current (CKSW), Shaunavon (CJSN), Moose Jaw (CHAB), Estevan (CJSL), Weyburn (CFSL), Rosetown/Kindersley (1330/1210), Lloydminster (CKSA) and Melfort (CJVR).
- **D.B. Fowler.** June 2, 2005 - Frost tolerant wheat - a) Manitoba Co-operator; b) Western Producer; c) CKLQ Brandon MB; d) Golden West Radio CFAM Altona MB; e) CJWW Saskatoon; f) CKRM Regina; g) CFCW Edmonton.
- **D.B. Fowler.** June 1, 2005 Frost-tolerant wheat to save farmers multi-millions. Western Grains Research Foundation, Saskatoon, Sask.
- **D.B. Fowler** and A. Laroche. June 28, 2004 – Winter wheat researchers search for cold hardiness "on/off switch". Western Grains Research Foundation.
- **R. Chibbar and D.B. Fowler.** June 9, 2004 – University of Saskatchewan – Swedish research into cereal crop quality. Western Producer.

G. PROJECT PHOTOS

High quality photos are included in the posters. If you require any other photos, please let us know.

H. ACKNOWLEDGEMENT

The project would like to thank Saskatchewan Agriculture, Food and Rural Revitalization for the financial support. We have acknowledged this support in all the FGAS project newsletters (http://www.genomeprairie.ca/abioticstress/media1/index.htm) and in all the posters and presentations developed by project researchers.

I. EXPENSE STATEMENT

The financial report will be provided by the University of Saskatchewan Financial Services in February 2006.

J. ICAR DATA ENTRY

The FGAS project proposal outline has been entered on the ICAR Inventory of Research website database under the ICAR ID 3333 1666.We will update our ICAR entry at the completion of the FGAS project at the end of March 2006.

Functional Genomics of Abiotic Stress (FGAS) at Genome Prairie

Proposed | Achieved | Projected

Year 1		Year 2				Year 3				Year 4				Year 5			
Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
							Aug-03	Nov-03	May-04	Jun-04	Sep-04	Dec-04	Mar-05	Jun-05	Sep-05	Dec-05	Mar-06

Wheat

i. Germplasm Development & Genomic Mapping — Mapping & Expression Populations; Field Validation

ii. Bin Mapping of Wheat Genes — 600

iii. BAC Construction and Characterization — 1,000,000

iv. EST Sequencing — 80,000 ESTs

v. Microarray Development — 10,000 spot array

vi. Proteomic Analysis — Identification of Cold Acclimation Proteins — Analyses Ongoing

vii. Transformation & Transgenic Plant Development — Analyses ongoing

viii. Low Temp Functional Genomics — Functional Studies,Transcription Factors, Promoter Isolation, Expression Profiling, etc. — Ongoing

ix. Bioinformatics — Updating

Brassica

i. Identification of Novel Genes For Freezing/Drought Tolerance
Identify genes induced/repressed by abiotic stress — Identification of differentially expressed genes

Role of select genes — 20 select genes — Updating

ii. Effect of Abiotic Stress on Gene Expression in NDI Cultures
Identify genes induced/repressed by abiotic stress — Identification of differentially expressed genes — Ongoing

Transformation and assessment of phenotype — Ongoing

iii. Proteomic and Genomic Analysis of Abiotic Stresses in Roots
Identify exudate and signalling proteins — 60-70 root exudate proteins; 150 signalling proteins — Ongoing

Identify novel genes & changes in gene expression related to nutrient stress — Microarray analysis; 120 hybridizations — Ongoing

Interactions between glycolytic enzymes & other proteins/enzymes — To identify interacting partners for 26 proteins

Modulation of protein-protein interactions by abiotic stress

Functional genomics of hypoxic stress — Analysis of transgenic plants for metabolic variation/analysis of regenerated whole plants under stress

iv. Elucidating the Role of ABA in Abiotic Stress

Identify & characterize novel genes coding for ABA binding proteins — Microarray Analysis (Approx 100 hybridizations)

To identify and characterize the ABA binding proteins — Ongoing

v. Tilling

Complete TILLING of 25 genes in *Arabidopsis*

To determine the feasibility of TILLING in *Brassica Oleracea*